DENTAL LIBRARY

cop. 2

Volume XIV Number 5

DEC 14 1934

cop. 2

JOURNAL of DENTAL RESEARCH

Official Publication of the
INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

October, 1934

On the increase in the diameters of the molar teeth of the rat after eruption. *Henry H. Donaldson*, Ph.D., D.Sc. (Wistar Institute of Anatomy and Biology)..... 323

Silver bromide as a pulp stain. *J. Staz*, L.D.S.; R.C.S., Eng. (University of the Witwatersrand)..... 347

Hardness study of transformations in gold-copper alloys. *Harry H. Asher*, D.D.S., M.S. (University of Illinois)..... 349

Studies, in the rat, of susceptibility to dental caries. II. Effects on teeth, in mothers and young, of diets deficient in calcium and vitamin D, during pregnancy, lactation, and rearing. *Theodor Rosebury*, D.D.S., and *Genevieve Foley*, M.A. (Columbia University)..... 359

Effects of oral treatment on the blood picture in aplastic anemia. *L. S. Pettit*, D.D.S. (Ohio State University)..... 379

Human saliva. I. Critical discussion of former salivary calcium studies and their value in the establishment of normal standards. *H. Becks*, Dr. med. et med. dent., D.D.S., and *W. W. Wainwright*, D.D.S., M.S. (University of California)..... 387

DENTAL LIBRARY
UNIVERSITY OF MICHIGAN
REMOVED

PUBLISHED BI-MONTHLY AT MT. ROYAL AND GUILFORD AVENUES, BALTIMORE, MD.
By THE JOURNAL OF DENTAL RESEARCH, Inc.
EDITORIAL OFFICE: 632 West 168th St., New York City
Made in United States of America

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

HONORARY OFFICERS (1934–'35). *Vice-Presidents*—Evelyn Sprawson, London. Hermann Wolf, Vienna. Jan Jesensky, Prague.

ACTIVE OFFICERS (1934–'35). *President*—J. L. T. Appleton, Jr., Univ. of Pennsylvania. *President-elect*—Theodore B. Beust, Univ. of Louisville. *Vice-president*—William G. Skillen, Northwestern Univ. *Treasurer*—Bissell B. Palmer, Fifth Avenue Hospital (New York City). *Secretary*—William J. Gies, Columbia University. *Trustees of the Endowment Fund*—The Treasurer; R. W. Bunting, Univ. of Michigan; A. D. Black, Northwestern Univ. *Acting Board of Editors*—The first five active officers.

THE SECTIONS—THEIR SECRETARIES, AND REPRESENTATIVES IN THE COUNCIL AND IN THE BOARD OF EDITORS (1934–'35)

(Asterisks indicate that representatives have not yet been selected)

Division	*Section*	*Secretary*	*Councillor*	*Editor*
Austria	Vienna	Fritz Driak	Balint Orban	Bernhard Gottlieb
Canada	Halifax	Hugh M. Eaton	G. K. Thomson	S. G. Ritchie
	Toronto	A. J. Broughton	A. J. McDonagh	A. J. McDonagh
	Winnipeg	E. Roy Bier	H. J. Merkeley	E. Roy Bier
China	Chengtu	R. Gordon Agnew	R. Gordon Agnew	R. Gordon Agnew
Czecho-slovakia	Prague	Friedr. Neumann	Friedr. Neumann	Jan Jesensky
England	London	Evelyn Sprawson	*	*
Hungary	Budapest	Ferenc Kösceg	Josef Szabo	Josef Szabo
S. Africa	Johannesburg	W. A. Wilson	*	J. C. M. Shaw
United States	Ann Arbor	U. G. Rickert	U. G. Rickert	U. G. Rickert
	Baltimore	M. S. Aisenberg	M. S. Aisenberg	A. H. Schultz
	Boston	Francis H. Daley	L. M. S. Miner	P. E. Boyle
	Chicago	James R. Blayney	V. T. Nylander	E. H. Hatton
	Cleveland	S. W. Chase	Thomas J. Hill	S. W. Chase
	Columbus	Paul C. Kitchin	Paul C. Kitchin	R. D. McFarland
	Louisville	Raymond E. Myers	T. B. Beust	T. B. Beust
	Minnesota	Carl W. Waldron	Carl W. Waldron	*
	New Haven	B. G. Anderson	B. G. Anderson	B. G. Anderson
	New York	Theodor Rosebury	L. M. Waugh	Theodor Rosebury
	Philadelphia	Thomas J. Cook	Thomas J. Cook	Carrie K. Bryant
	Pittsburgh	L. E. Van Kirk	Kaethe Dewey	L. E. Van Kirk
	Richmond	J. C. Forbes	Harry Bear	J. C. Forbes
	Rochester	G. Van Huysen	H. J. Sedwick	H. C. Hodge
	San Francisco	W. B. Ryder	J. A. Marshall	J. A. Marshall
	St. Louis	C. O. Simpson	Geo. B. Winter	R. C. Wheeler
	Washington	G. C. Paffenbarger	Wilmer Souder	H. E. Harvey

SOCIETIES THAT ACTIVELY SUPPORT THE JOURNAL OF DENTAL RESEARCH, AND THEIR REPRESENTATIVES IN THE HONORARY BOARD OF EDITORS

First District Dental Society of the State of New York ..... JOHN OPPIE MCCALL
New York Academy of Dentistry..... THEODOR ROSEBURY
American College of Dentists..... ALBERT L. MIDGLEY, Providence
Academy of Stomatology (Philadelphia) ..... JOHN BURKHARDT, JR.
Allied Dental Council (New York) ..... LEON HARRIS
American Academy of Dental Science..... CHARLES E. HATCH, Boston
American Stomatological Association:
H. J. LEONARD AND EDWARD FRANKEL, JR., New York City
Kings County (N. Y.) Dental Society ..... LEONARD KOHN
Odontographic Society (Chicago)..... HOUSTON F. METHVEN
Robert R. Andrews Society for the Promotion of Research (Tufts).... CHARLES E. HATCH

Copyright, 1934, by The Journal of Dental Research, Inc.
Entered as second-class matter June 24, 1919, at the Post Office at Baltimore, Maryland, under the act of March 8, 1879

ON THE INCREASE IN THE DIAMETERS OF THE MOLAR TEETH OF THE RAT AFTER ERUPTION[1]

HENRY H. DONALDSON, PH.D., D.Sc.

Wistar Institute of Anatomy and Biology, Philadelphia, Pa.

In 1927 Donaldson and French (2) presented data which they interpreted as showing post-eruptive growth in the diameters of the molar teeth of the albino rat. In 1931 Wood and Wood (5) interpreted the data which they obtained as showing that these teeth did not grow after eruption. This refutation has led me to make a fresh study of the problem. The results show that post-eruptive growth occurs in these teeth.

The dental formula for the teeth of the rat is:

$$I\frac{1}{1}\,C\frac{0}{0} - P\frac{0}{0}\,M\frac{3}{3}$$

The appearance of the rows of molars is shown in *fig. 1*. The symbol M is used to designate a molar tooth—this followed by the number of the tooth. The numbering is 1, 2, 3, from before backward in each jaw. For maxillary molars, M is underlined; for mandibular molars, overlined. Where M is used without lines, it refers to the same tooth in both jaws. M1–3 indicates length of the entire row of three molars in both jaws. The usual time of eruption is: M1, 19th day; M2, 21st day; M3, 35th day (Donaldson: "The Rat," '24).

In the present study, the youngest groups were measured at 37 days of age. All measurements were made on both sides of each jaw. Determinations were made in both jaws (a) on the length of the rows, measuring between the points indicated in *fig. 1*; (b) on the length of M1, taken between the extreme edges of that tooth; (c) and on

[1] Presented at the twelfth general meeting of the International Association for Dental Research, Chicago, Ill., March 17–18, 1934; *J. Den. Res.*, 1934, *14*, 158. The cost of the illustrations has been met from a grant, to the *Journal*, by the Academy of Stomatology of Philadelphia. The Academy's generous support is appreciated by the author and by the editors.—[*Ed.*]

the width of each of the three teeth in each jaw. Width was the greatest distance between the sides of the tooth, taken at right angles to its long axis. All measurements were made with a comparator. This instrument consists of a microscope magnifying 5 diameters, and carrying cross hairs in the eye-piece. The microscope is fitted to a plate that slides in a frame bearing a millimeter scale. The

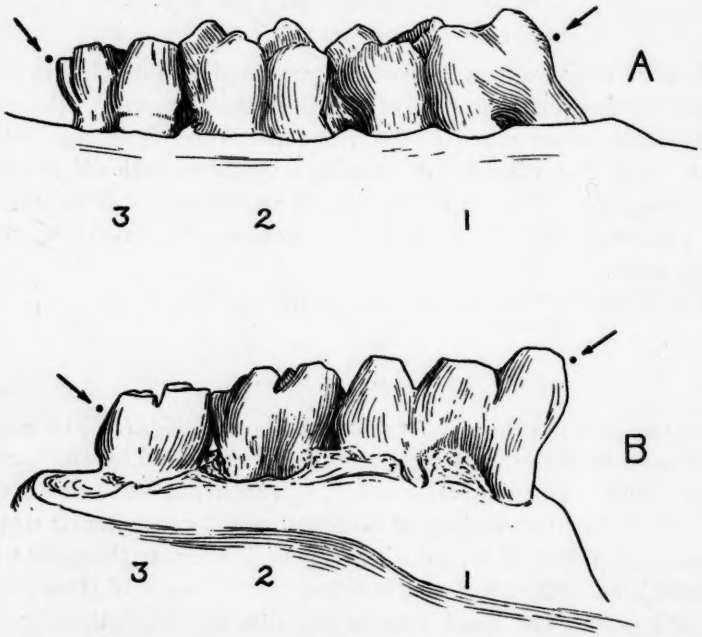

FIG. 1. Rows of rat molars: *A* and *B*; male, age 205 days. To illustrate points from which measurements were taken for length of rows. Arrows indicate points used in each case. *A*, maxillary row (right); palatal aspect. *B*, mandibular row (left); palatal aspect.

position of the plate is controlled by a fine micrometer-screw. One turn of the head of the screw moves the plate 1 mm. The screw head is marked in hundredths of a millimeter, but as the space between successive marks is almost 2 mm., it is possible to estimate thousandths of a millimeter with fair accuracy. For measurements of teeth in the cranium or rami, these were fixed with soft cement in the proper position on a slide, and measured, by the aid of the micrometer screw,

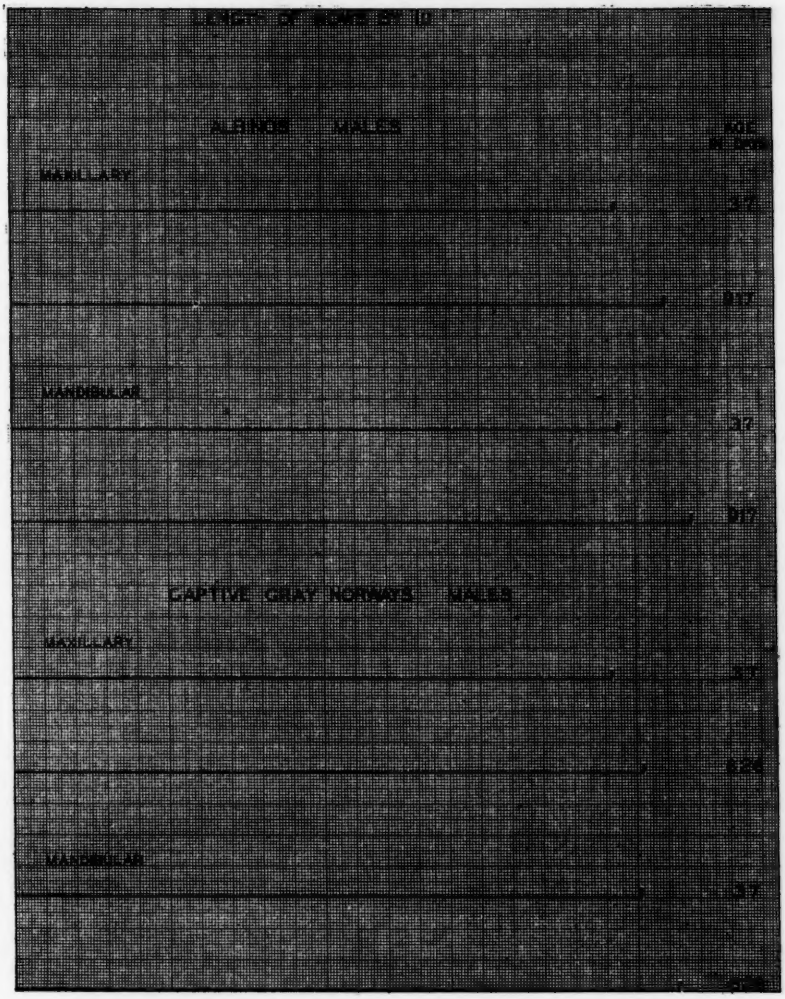

FIG. 2. Chart showing, in male albinos and male captive gray Norways, lengths, by 10, of maxillary and mandibular rows at 37 days of age, compared with respective lengths in oldest groups. Data from *tables 1* and *2*.

at a magnification of 5 diameters under the microscope of the comparator. All measurements were recorded to thousandths of a milli-

meter; for computation, three decimal places were used. The observed values for right and left sides were averaged. For convenience, values are given to only two decimal places in all cases, except for the width of individual teeth. Tests show that an error of ±0.01 mm.

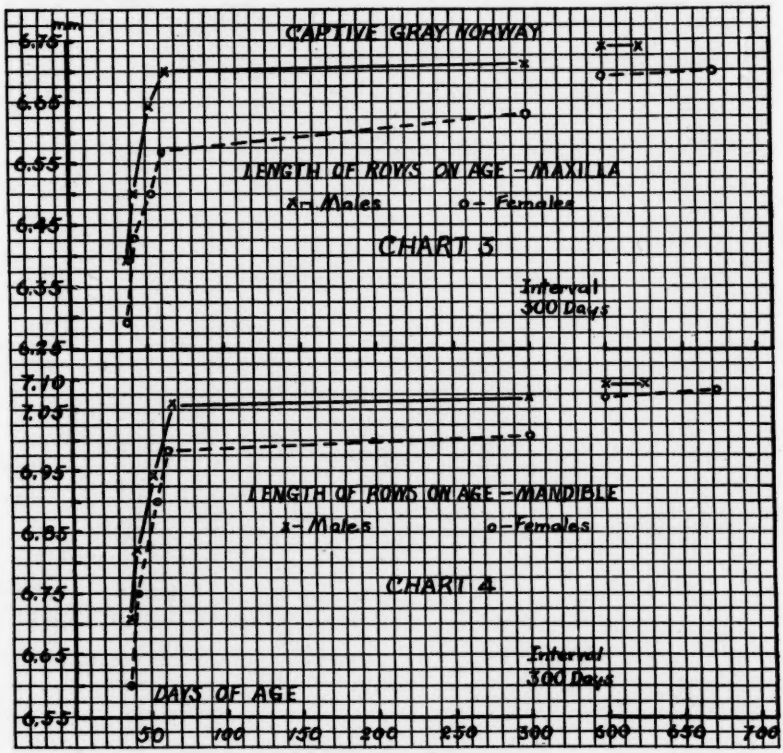

FIG. 3. Length of rows, on age: M1–3. *Chart 3:* captive grays; maxilla. Males x———x; females o-------o. *Chart 4:* captive grays; mandible. Males x———x; females o-------o.

may occur in individual measurements, due mainly to difficulty in applying the cross hairs to exactly the same spot on the specimen. These errors are, of course, most significant in the case of the smaller values, such as those for the width of teeth, but in no instance do they essentially disturb the relations found. The teeth were meas-

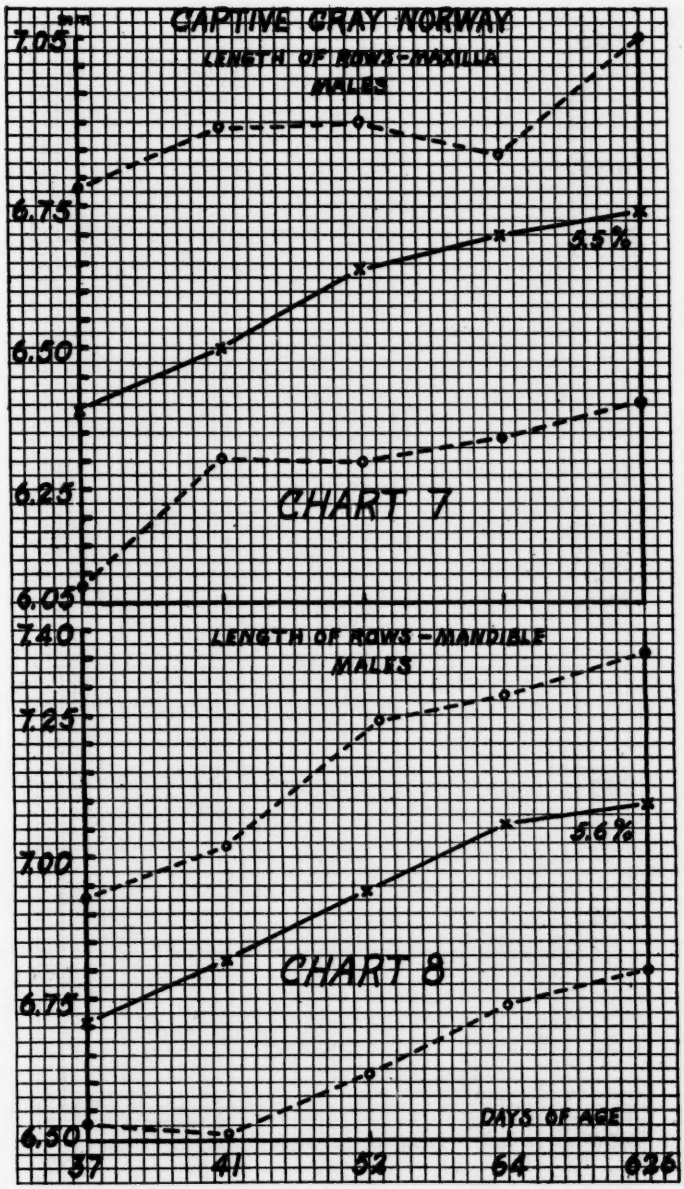

FIG. 4. Length of rows: M1–3; equal intervals. *Chart 7:* captive grays; males; maxilla. *Chart 8:* captive grays; males; mandible. o-------o—extreme values within each age group; entered in each chart, 4–52.

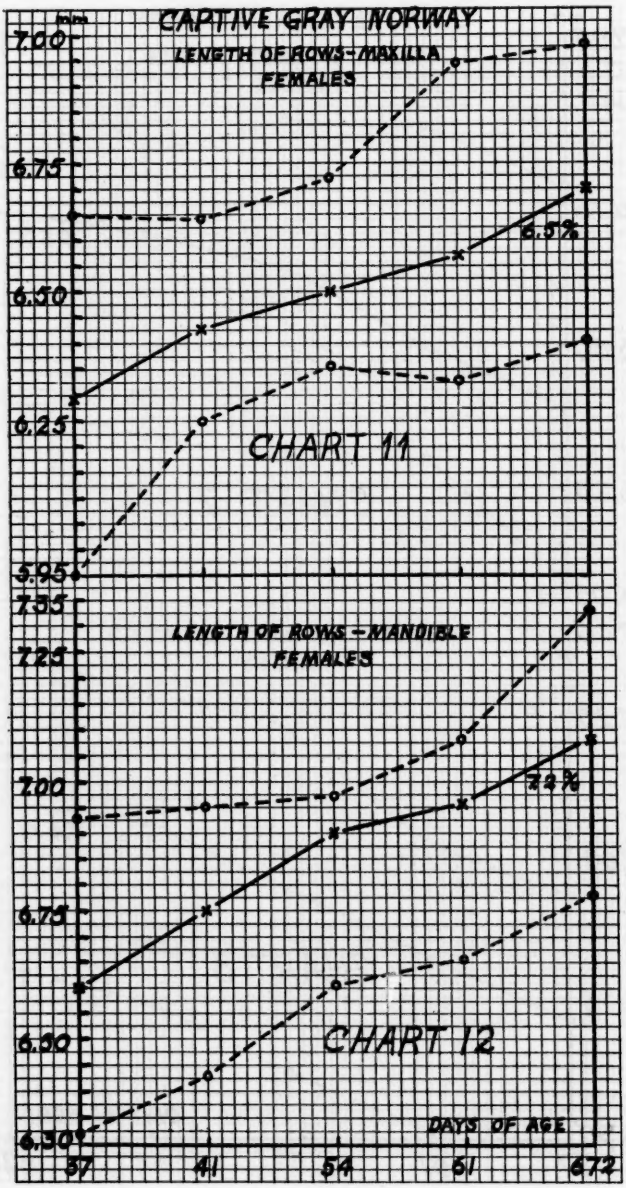

FIG. 5. Length of rows: M1–3; equal intervals. *Chart 11:* captive grays; females; maxilla. *Chart 12:* captive grays; females; mandible.

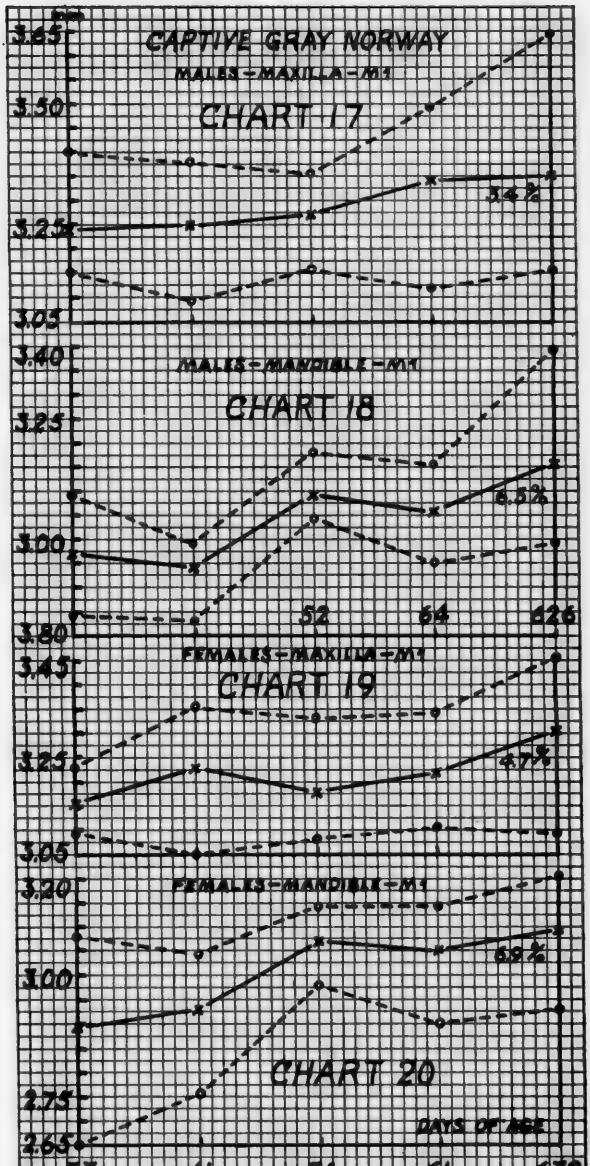

FIG. 6. M1: length. *Chart 17:* captive grays; males; maxilla. *Chart 18:* captive grays; males; mandible. *Chart 19:* captive grays; females; maxilla. *Chart 20:* captive grays; females; mandible.

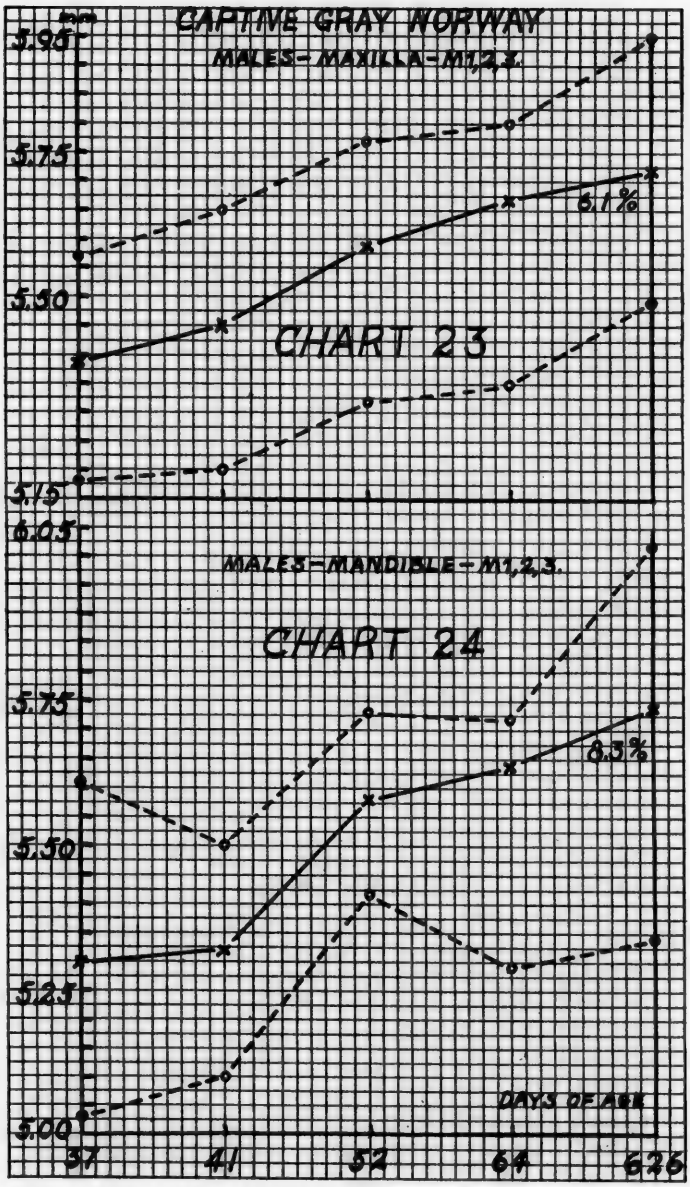

FIG. 7. Total width of teeth: M1 + M2 + M3. *Chart 23:* captive grays; males; maxilla. *Chart 24:* captive grays; males; mandible.

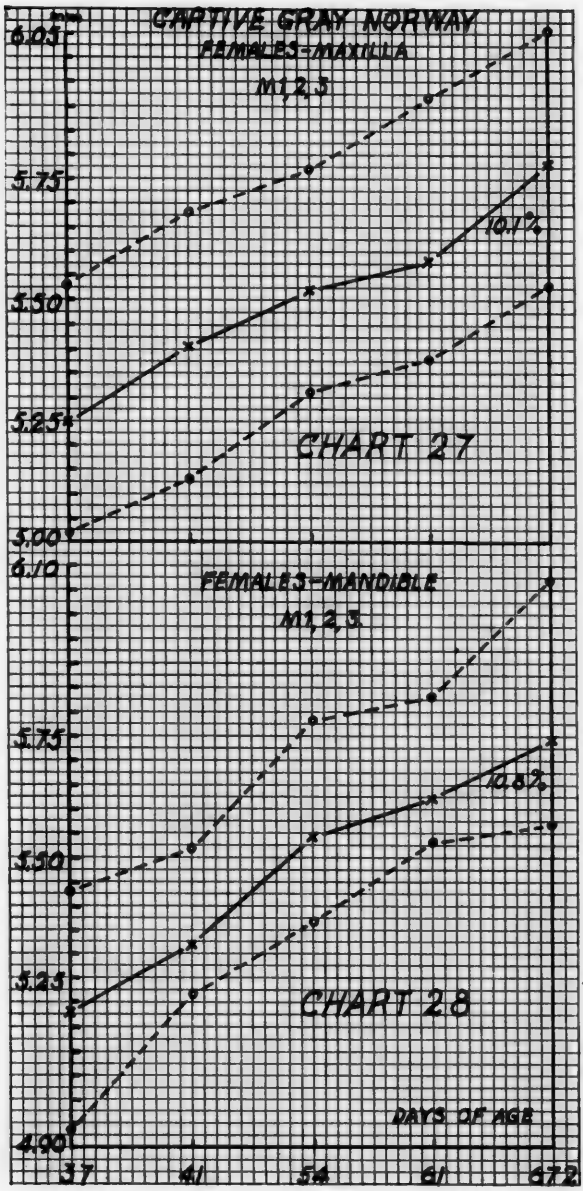

FIG. 8. Total width of teeth: M1 + M2 + M3. *Chart 27:* captive grays; females; maxilla. *Chart 28:* captive grays; females; mandible.

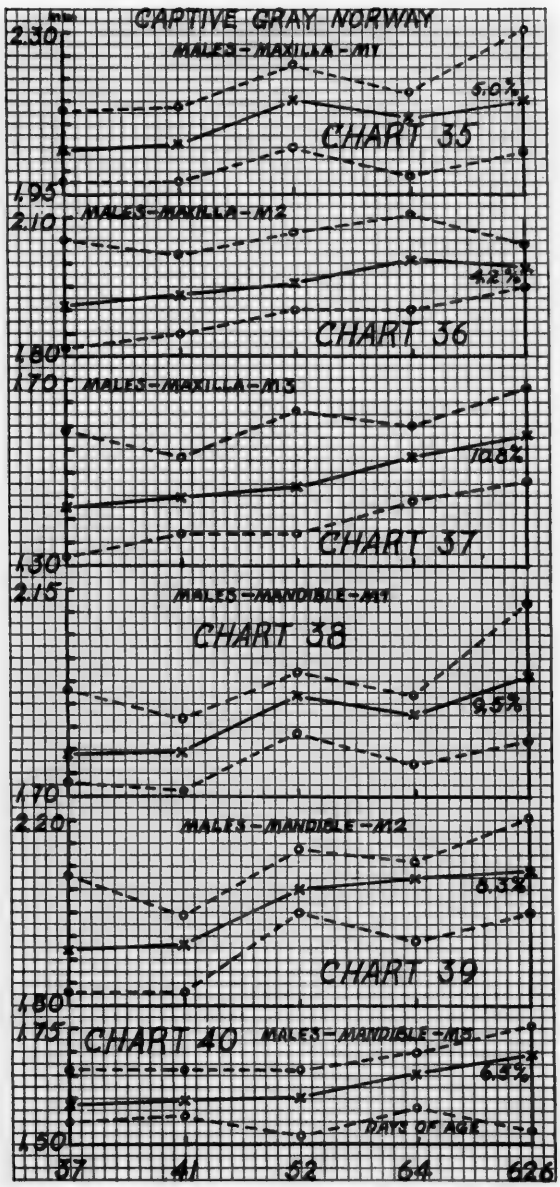

FIG. 9. Width of individual teeth. *Charts 35, 36, 37:* captive grays; males; M1, M2, M3—maxilla. *Charts 38, 39, 40:* captive grays; males; $\overline{M1}$, $\overline{M2}$, $\overline{M3}$—mandible.

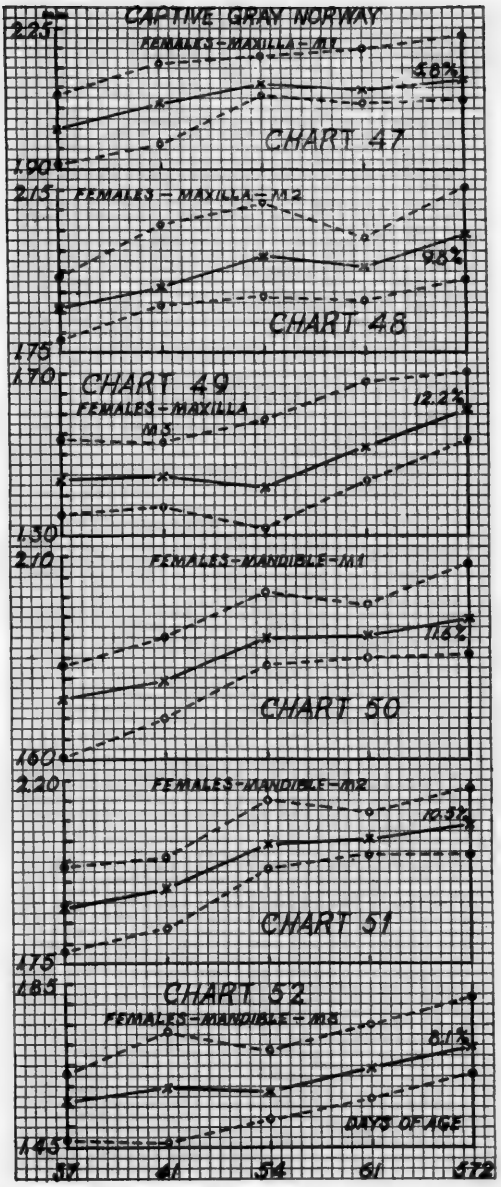

FIG. 10. Width of individual teeth. *Charts 47, 48, 49:* captive grays; females; M1, M2, M3—maxilla. *Charts 50, 51, 52:* captive grays; females; $\overline{M1}$, $\overline{M2}$, $\overline{M3}$—mandible.

TABLE 1

A. Albinos: length of rows, M1–3

	AGE	BD. WT.	BD. L.	MAXILLARY	MANDIBULAR
	days	*gms.*	*mm.*	*mm.*	*mm.*
Males	37	86	150	6.46	6.84
	60	162	184	6.79	7.03
	262	343	236	6.86	7.18
	*300**	...	...	*6.87*	*7.19*
	*900**	...	...	*6.99*	*7.24*
	917	362	234	7.01	7.26
(Gain)				8.6%	6.1%
Females	37	76	142	6.36	6.76
	60	121	166	6.69	7.02
	273	274	215	6.73	7.01
	*300**	...	...	*6.74*	*7.02*
	*900**	...	...	*6.87*	*7.06*
	949	249	214	6.89	7.17
(Gain)				8.3%	6.0%

B. Albinos: length of M1

	AGE	MAXILLARY $\underline{M1}$	MANDIBULAR $\overline{M1}$		AGE	MAXILLARY $\underline{M1}$	MANDIBULAR $\overline{M1}$
	days	*mm.*	*mm.*		*days*	*mm.*	*mm.*
Males	37	3.38	3.00	*Females*	37	3.29	2.99
	60	3.41	3.01		60	3.38	2.98
	262	3.43	3.08		273	3.35	3.05
	917	3.46	3.20		949	3.36	3.15
(Gain)		2.4%	6.6%			2.1%	5.3%

C. Albinos: total width of M1 + M2 + M3[1]

	AGE	MAXILLARY $\underline{M1, 2, 3}$	MANDIBULAR $\overline{M1, 2, 3}$		AGE	MAXILLARY $\underline{M1, 2, 3}$	MANDIBULAR $\overline{M1, 2, 3}$
	days	*mm.*	*mm.*		*days*	*mm.*	*mm.*
Males	37	5.94	5.57	*Females*	37	5.82	5.55
	60	5.98	5.69		60	5.92	5.66
	262	6.00	5.72		273	5.94	5.67
	917	6.15	5.93		949	6.03	5.84
(Gain)		3.5%	6.5%			3.6%	5.2%

D. Albinos: width of individual teeth

	AGE	MAXILLARY			MANDIBULAR		
		$\underline{M1}$	$\underline{M2}$	$\underline{M3}$	$\overline{M1}$	$\overline{M2}$	$\overline{M3}$
	days	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*
Males	37	2.133	2.099	1.708	1.869	2.019	1.698
	60	2.165	2.099	1.696	1.894	2.058	1.694
	262	2.172	2.099	1.728	1.942	2.078	1.716
	917	2.213	2.177	1.776	2.000	2.134	1.794
(Gain)		3.7%	3.7%	4.0%	7.0%	5.7%	5.6%
Females	37	2.113	2.050	1.652	1.850	2.013	1.682
	60	2.129	2.097	1.692	1.909	2.062	1.709
	273	2.155	2.104	1.700	1.922	2.058	1.687
	949	2.163	2.124	1.745	1.965	2.129	1.742
(Gain)		2.3%	3.6%	5.6%	6.2%	5.2%	3.5%

* Interpolation for adjustment of graph.

[1] Based on data in section D of this table.

TABLE 2

A. Captive gray Norways: length of rows, M1–3

	AGE	BD. WT.	BD. L.	MAXILLARY	MANDIBULAR
	days	*gms.*	*mm.*	*mm.*	*mm.*
Males	37	63	135	6.39	6.71
	41	78	144	6.50	6.82
	52	86	153	6.64	6.94
	64	129	173	6.70	7.06
	*300**	...	...	*6.71*	*7.07*
	*600**	...	...	*6.74*	*7.09*
	626	400	240	6.74	7.09
(Gain)				5.5%	5.6%
Females	37	60	130	6.29	6.60
	41	73	141	6.43	6.75
	54	83	148	6.50	6.90
	61	100	158	6.57	6.96
	*300**	...	...	*6.63*	*7.01*
	*600**	...	...	*6.69*	*7.07*
	672	318	226	6.70	7.08
(Gain)				6.5%	7.2%

B. Captive gray Norways: length of M1

	AGE	MAXILLARY $\underline{M1}$	MANDIBULAR $\overline{M1}$		AGE	MAXILLARY $\underline{M1}$	MANDIBULAR $\overline{M1}$
	days	*mm.*	*mm.*		*days*	*mm.*	*mm.*
Males	37	3.24	2.97	*Females*	37	3.16	2.89
	41	3.25	2.94		41	3.23	2.93
	52	3.27	3.09		54	3.18	3.07
	64	3.34	3.06		61	3.22	3.05
	626	3.35	3.16		672	3.31	3.09
(Gain)		3.4%	6.5%			4.7%	6.9%

C. Captive gray Norways: total width of M1 + M2 + M3[1]

	AGE	MAXILLARY $\underline{M1, 2, 3}$	MANDIBULAR $\overline{M1, 2, 3}$		AGE	MAXILLARY $\underline{M1, 2, 3}$	MANDIBULAR $\overline{M1, 2, 3}$
	days	*mm.*	*mm.*		*days*	*mm.*	*mm.*
Males	37	5.39	5.30	*Females*	37	5.25	5.18
	41	5.45	5.32		41	5.41	5.32
	52	5.59	5.58		54	5.52	5.54
	64	5.67	5.64		61	5.58	5.62
	626	5.72	5.74		672	5.78	5.74
(Gain)		6.1%	8.3%			10.1%	10.8%

D. Captive gray Norways: width of individual teeth

		MAXILLARY			MANDIBULAR		
	AGE	$\underline{M1}$	$\underline{M2}$	$\underline{M3}$	$\overline{M1}$	$\overline{M2}$	$\overline{M3}$
	days	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*
Males	37	2.047	1.912	1.427	1.793	1.925	1.586
	41	2.062	1.936	1.449	1.796	1.931	1.596
	52	2.155	1.961	1.471	1.922	2.057	1.603
	64	2.118	2.016	1.531	1.905	2.076	1.656
	626	2.150	1.988	1.582	1.964	2.085	1.690
(Gain)		5.0%	4.2%	10.8%	9.5%	8.3%	6.5%
Females	37	2.003	1.859	1.438	1.745	1.893	1.567
	41	2.066	1.912	1.448	1.792	1.929	1.598
	54	2.111	1.987	1.422	1.901	2.045	1.585
	61	2.095	1.960	1.519	1.909	2.063	1.648
	672	2.121	2.041	1.614	1.948	2.093	1.694
(Gain)		5.8%	9.8%	12.2%	11.6%	10.5%	8.1%

* Interpolation for adjustment of graph.

[1] Based on data in section D of this table.

ured in skulls that had been cleaned in "Gold Dust Washing Powder," and then dried at 95°F. This treatment causes a shrinkage of 0.05 mm. in the length of the rows, and 0.012 mm. in the width of the teeth, but such slight shrinkage is similar in both young and old skulls, and does not, therefore, have a differential effect.

Under the conditions just described, measurements were made on the teeth of 40 male and 40 female albino rats from the Wistar Institute Colony, taken in groups of ten, at the ages indicated in *table 1*. Similarly, on 50 males and 50 females, from Dr. King's Colony of captive gray Norways at the Wistar Institute—from the 11th to the 15th generation in captivity—at the ages for the five groups of ten given in *table 2*. These two tables contain the data for the four forms of measurement that were made. To facilitate comparison of the corresponding data for the two strains, the data are placed on opposite pages. In *tables 1* and *2*, mean body-weights and body-lengths for each group of ten are the same for each section (A–D).

Interest centers, in each set of measurements, in the comparison of values for the oldest group with those at 37 days—so the percentage excess for the oldest group is given immediately under the absolute value for that group. It will be noted that the values for the oldest groups are in every case above the corresponding values at 37 days, and in every instance the values given for the percentage difference are statistically valid. To illustrate age differences in the lengths of the rows here considered, the chart on p. 325 (*fig. 2*) shows for albinos the absolute lengths × 10 of the maxillary and mandibular rows at 37 and 917 days, respectively, and similarly in the captive grays at 37 and 626 days. The differences thus shown are clearly evident.

Charts based on the data in *tables 1* and *2* were numbered from 1 to 52. The graphs for the albinos and for the captive grays are in close agreement. Since those for the captive grays include more records for early ages, it has been decided to limit the presentation to those for the captive grays alone. Thus only data in *table 2* are here represented by graphs. These bear the numbers 3, 4, 7, 8, 11, 12, 17–20, 23, 24, 27, 28, 35–40, 47–52 (*figs. 3–10*). To show the age relations of the growth change, graphs 3 and 4 (*fig. 3*), giving the values for the length of the rows, are charted up to 300 days, followed by the final values for the oldest groups, which are displaced.

These graphs serve to show the rapid change up to 60 days. In view of the inconvenience of this method of charting, the remaining graphs are presented in a more condensed form. In this latter form of chart, the values for each group are entered at equal intervals. This form readily permits a record of the extreme values within each group, and these are also shown in each instance by the graphs in broken lines.[2] In successive groups the values for the maxima and minima for the most part run as do those for the averages, and the averages in turn tend to approximate the means. Despite considerable variability within each age-group, these relations indicate a fairly similar distribution within the several age-groups. The individuals forming each group are, so to speak, in company formation, and the whole company tends to move to a higher level with advancing age.

If we are dealing with regular growth changes, groups intermediate in age between the extremes should yield intermediate values. For the albinos, in a total of 24 graphs (not given here), 16 show, with advancing age, a regular progressive change in the mean values. In 6 graphs one value is out of order; and in 1 graph, two values. These relations are shown in the corresponding tables. For the captive grays—with five entries—in a total of 24 graphs, 14 show a regular progressive change; in 9 graphs, one value is out of order; and in 1 graph, two values. In the cases where values are out of order for age, the departures are in general slight. The captive Norways are less regular than the albinos in these relations, but they carry one more entry—hence a greater chance for irregularity.

RESULTS

Absolute values. In an examination of the tables and charts just given, we find that in every case (i.e., in both sexes of both strains) the values for the oldest groups are statistically above those for 37-day groups, and that of the total of 216 entries ($24 \times 4 = 96$ and $24 \times 5 = 120$) only 9 percent are out of order. It is concluded that, after 37 days, the various values determined for these teeth increase in each strain with age, and that therefore these teeth grow in diameter after eruption. This is the outstanding result. There are, however,

[2] By presenting the limiting values in this manner, it becomes unnecessary to give them in tabular form.

several minor relations among the data that seem worth consideration here, since in some instances they point to regularities which enhance the trustworthiness of the observations.

According to sex. In the albinos, in every instance—excepting the 60-day group—for the width of $\overline{M}3$, absolute values for the females are less than those for the males of like ages. In the captive gray Norways, absolute values for the females are, without exception,

TABLE 3

Final percentage gains

	ALBINOS		CAPTIVE GRAYS	
	Max.	Mand.	Max.	Mand.
Total length: M1–3				
Males	8.6	6.1	5.5	5.6
Females	8.3	6.0	6.5	7.2
Length: M1				
Males	2.4	6.6	3.4	6.5
Females	2.1	5.3	4.7	6.9
Total width: M1 + M2 + M3				
Males	3.5	6.5	6.1	8.3
Females	3.6	5.2	10.1	10.8
Width: Individual teeth				
Males: M1	3.7	7.0	5.0	9.5
M2	3.7	5.7	4.2	8.3
M3	4.0	5.6	10.8	6.5
Sums	11.4	19.2	20.0	24.3
Females: M1	2.3	6.2	5.8	11.6
M2	3.6	5.2	9.8	10.5
M3	5.6	3.5	12.2	8.1
Sums	11.5	14.9	27.8	30.2

less. Thus the female gives lower values than does the male. The basis for this relation will be considered later.

According to strain. At corresponding ages, absolute values for the captive grays are less than those for the albinos in 47 of the 50 comparisons possible. The nonconforming cases are all for the length of $\overline{M}1$—in one case for males; in two for females (*table 1*, B).

Percentage values. *Table 3* gives the percentage gains shown by the oldest groups of both strains, when they are compared with the initial groups at 37 days. Before attempting any comparisons of

data in *table 3*, it should be noted that the percentage gains among the albinos are based on values for old rats, respectively 917 and 949 days of age, while those for the captive grays are on old rats only 626 or 672 days of age. For exact comparison, the data for the albinos should therefore be reduced to those for the ages shown by the captive grays. This matter has been considered. It appears, in the first place, that the reductions thus brought about in the percentages for the albinos are slight. Moreover, such reductions do not alter any of the relations to which attention will be called; and hence, to avoid the introduction of an extra set of values for the albinos, no changes in the observed albino values have been made. In all the subsequent comparisons made by strain, the albino values are taken as the standard—and for sex, the male values.

Comparison according to strain: percentage values. In length of rows: in three out of the four comparisons, the percentages for the captive grays are low (*table 3*). In M1 length, in total width, and in width of individual teeth (using sums), the percentages for the captive grays are distinctly high. The most marked difference between the two strains is in the greater increase in width of the teeth shown by the captive grays.

Another relation which calls for comment is the order of the percentages for width of individual teeth, from M1 to M3. In the maxilla, the percentage increases from M1 to M3, in both sexes and both strains. The sequence is perfect in the females, but in the males of both strains M2 is out of order. In the mandible the order of percentages is reversed, but here the sequence is perfect in both sexes of both strains.

Maxilla and mandible: percentage values. In the length of the rows for the albino, the percentage values for the mandible are, in both sexes, less than those for the maxilla. In all the other instances, for both albinos and captive grays in *table 3* (using the sums for the individual teeth), the percentage values in the mandible are above those for the maxilla.

According to sex. In the albinos, save in the width of the maxillary teeth, the percentages for females are always *below* those for males. In the captive grays, the percentages for females are, in every instance, above those for males.

According to locality. In the case of M1 length, the percentage gain in the albinos is small, but in the captive grays relatively much larger. Relation of M1 length to length of row: if the percentage increase in length of M1 is approximately the same as that for the entire row, then the remaining teeth, M2 and M3, taken together, will have increased in length as does M1. If, however, the increase for M1 is below that for the entire row, then the remaining teeth must contribute more to the increase in the length of the row. The data in *table 3* show that in both strains, $\overline{M1}$ (i.e., for the mandibles) increases in length approximately as do the respective rows, while in the case of $\underline{M1}$ (i.e., for the maxilla) the increase in length is less; and in the albino, much less than that in the corresponding rows. In these latter instances, therefore, M2 and M3 contribute most to the increase in the length of the rows.

In summary: percentage values. According to strain, the percentage values in the captive grays are high, save in the case of length of rows, and especially high for width of teeth. As compared with the maxillary, the mandibular values are consistently high, while the percentages for females are consistently higher than those for males. Also, the values for $\overline{M1}$ length are high, and nearer those for the length of the rows.

This general excess in percentage values for the captive grays should be considered in the light of absolute values for the several measurements involved. At 37 days absolute values, both initial and final, for the captive grays, are below those for the albinos, by various amounts. In general, initial values are relatively lower than final values; and, in the case of values for width, are much lower than those for the albinos. It is due to these latter relations that the captive grays give the high percentage values for growth in the width of the teeth. Despite the higher percentage values in the case of the captive Norways, they nevertheless have the smaller teeth, an unexpected relation.

Width: percentage values for individual teeth. To illustrate further the manner in which these teeth grow in width, the percentage values for width of each of the three teeth on the sum of the widths has been determined. For the age-groups within both sexes of both strains, these percentages are highly constant. In *table 4* are given the aver-

ages for all age-groups for each sex of each strain. In both strains the averages according to sex are very similar. As to strain, the chief difference is that in the maxilla of the captive grays, M1 gives a high value, and M3 a low value. There is, therefore, a slight difference according to strain in the relative width of the teeth. It appears, however, that the increase in width of the teeth takes place in an orderly manner.

TABLE 4

Percentage of total width represented by width of each molar tooth

	MAXILLA			MANDIBLE		
	M1	M2	M3	M1	M2	M3
Albinos						
Male	36.1	35.2	28.7	33.7	36.2	30.1
Female	36.1	35.4	28.5	33.6	36.3	30.1
Captive grays						
Male	37.8	35.3	26.9	34.0	36.4	29.6
Female	37.4	35.4	27.0	33.9	36.5	29.6

DISCUSSION

The question under discussion is whether the molar teeth of the albino rat grow in length and width after eruption. In the previous study by Donaldson and French (3), it was found that such growth occurs. The data for that conclusion are given in tables 1 and 3 and in the graphs on age, in charts 5, 6, 7 and 8 (loc. cit.). The lengths of the rows were found to increase from 9 to 10 percent, between 48 and 496 days. On comparing the data in those tables with the data here presented, it appears that the former, excepting those for the male mandible, are based on notably low initial values and high final values. It is the low initial values that are responsible for the higher percentage gains. The measurements were made with dial calipers reading to 0.1 mm. It must be admitted with regret that this method of measurement was not fine enough for the purpose.

These results have been criticised by Wood and Wood (5). Their objections may be given briefly, with our comments on them. Wood

and Wood suggest that our results may have been in part accidental, as the age-groups contained only from 6 to 10 individuals. We have found, in the present study, that data from groups of 10 give fairly consistent results, but groups of 6 were certainly small. They suggest, further, that selection may have modified the constitution of the age-groups. In the present study the individuals forming a group were selected from among normal animals, according to age, not by myself, but by another person unacquainted with the purpose for which the rats were to be used. That animals with large teeth did not appear in the younger groups is shown by the graphs for the maximum values within each group, in charts 7–52. This same explanation applies to a possible selection through death of the younger animals with smaller teeth at earlier ages. In this connection, it may be pointed out that during the first 60 days—in the present study—growth of the teeth was most marked, though mortality was almost negligible; while after 260 days—during which period mortality is significant, due to lung infection, etc.—growth was much more restricted.

The teeth forming the rows are always aligned and contiguous in normal rats. Wood and Wood found no change in position in their rats, but we noted, in the old rats, a slight twist in M1, throwing it a trifle out of line. So far as this goes, it tends to shorten the measurement for the length of the row. No change of position has been noticed that would increase the length of the row in the older rats.

The paper by Donaldson and French (3) was criticised for not containing the range of values in the age-groups. This criticism was justified. The omission was in part remedied in the paper by Donaldson (2). The late appearance of the higher values was cited by Wood and Wood as an argument against our conclusions. This situation troubled the authors at the time, and the criticism was pertinent. The results in the present paper rectify the situation, and show, as would be expected, that rapid growth of the teeth comes during the first months. The probable explanation of the earlier results lies in the lack of refinement in those measurements.

It was urged by Wood and Wood that, as the enamel does not grow, the teeth cannot grow. This objection begs the question, and need not be considered.

Turning from the criticisms in the paper by Wood and Wood, we pass to the data which they have presented, on the basis of which they conclude that the molar teeth of the albino rat do not grow after eruption. They measured the teeth in the same animal at different ages. This is certainly the ideal procedure, if it can be carried out with precision. For measuring they used specially designed and accurate dial-gauge calipers, reading to 0.01 mm. In all, the teeth in four male and three female albinos were measured. In the living animals two methods of measurement, "direct" and from "impressions," were employed. The latter method was used the more frequently; hence the comparisons that follow are based on the "impression measurements" alone, since the values obtained by different methods of measurement, taken at different ages, are not comparable. Their data are given in six tables. In three animals only, designated A, B and H, were the first impressions taken at an early age: A and B at 47 days; H, at 21 days. Only the records for these three rats are considered here.

In *table 5* are given the percentage differences of the values obtained by Wood and Wood at the earliest and the latest ages. Where the measurements were made on both right and left sides, the mean value for the two sides is used. Values for only three characters are available—M1-3 (length of maxillary row), M1 length, and M1 width. The initial values are followed by H when high, and L when low in comparison with our data at like ages. In all, eight comparisons are made here. Five show a greater value in the old animal, and three a lesser value. The percentages indicate the amount of difference. When the relations shown in *table 5* are viewed in the light of the conclusions reached by Wood and Wood, that the molar teeth do not grow after eruption, they indicate too great a variability as between +3.7% and −3.3% to warrant such a conclusion. Wood and Wood did not analyze their data. The difference between our consistently plus results and those of Wood and Wood—sometimes plus and sometimes minus—depends, in some measure at least, on the high initial values which they obtained, indicated by H in *table 5*. In the one instance (A; M1 width) where the initial value was low (L), the final value was also very low, thus giving but a slightly plus percentage. From our point of view, therefore, the method used by Wood and

Wood tended to give high initial values, and thus to obscure any growth change with advancing age.

TABLE 5

Based on data from Wood and Wood ('31)

Table (loc. cit.)		V	I	II
Column		1	2	3
Rat	Age	M1–3	M1 length	M1 width
	days	mm.	mm.	mm.
A ♀	47	6.93 H	3.58 H	2.09 L
	709	7.08	3.53	2.11
Difference		+2.1%	−1.5%	+0.9%
B ♀	47	6.65 H	3.60 H	2.14 H
	709	6.90	3.61	2.16
Difference		+3.7%	+0.3%	+0.9%
H ♂	21	...	3.54 H	2.17 H
	561	...	3.45	2.10
Difference			−2.6%	−3.3%

TABLE 6

Percentage deviations from normal for teeth of runts, at 37 days of age

	AT 37 DAYS OF AGE		PERCENTAGE DEFICIENCY OF VALUES FOR RUNTS		
	Bd. wt.	Bd. l.	M1–3	M1 length	M1 width
	gms.	mm.			
Males:					
Normals	86	150			
Runts	46	121	−1.0	−2.3	−5.6
Females:					
Normals	76	142			
Runts	45	122	−2.1	−6.6	−9.2

Influence of body size on size of teeth. We have used age for the grouping of our rats. With increasing age, however, goes increasing

body-size, and it has therefore seemed desirable to examine the influence of body-size on the size of the teeth at like ages. Happily, Wood and Wood made measurements of M1–3, M1 length, and M1 width, on a runt, designated Z, at 155 days of age. These measurements can be best compared with those on rat A (*table 5*), though A was distinctly younger than the runt. In the runt the respective percentage deficiencies for the characters just named were −17, −16, and −9. Thus, this runt had teeth of small size. Unfortunately, we do not know the weight of this rat at 155 days of age. Following this suggestion of Wood and Wood, we made all the tooth measurements on a group of 10 male albino runts at 37 days of age, and, similarly, on a group of 10 females at 37 days of age. The percentage deviations for the runts from the normals of like age are given in *table 6*. Again the runts show smaller teeth than the normals, when the comparison is made at 37 days of age. No direct comparison can be made between our observations and those of Wood and Wood, because we have not the body-size of their animal, nor are there any data on the change in tooth-size with age in the case of runts. The two sets of observations merely show that runts have small teeth for their age.

The test for the effect of body-size has been applied, to our data for normal rats, by determining the value for the character measured in the heaviest four and in the lightest four, in each group of 10 rats, examining both sexes in both strains. In every instance, in a given strain, the larger rats show the higher values. Thus, at like ages, the teeth are larger in the bigger rats. Computation shows that between 37 and 60 days, when growth of both body and teeth is rapid, the length of the rows increases about 0.01 mm. for each millimeter increase in body-length, and about 0.005 mm. for each gram of increase in body-weight. After this age, however, the effects of body-size diminish so rapidly that, in the case of the oldest groups in each strain, they are negligible. On comparing the values for the length of the rows in the albinos with those for the captive grays, it is found that for the first 60 days the lower values for the captive grays are due to their smaller body-size, but, in the older groups, body-size has ceased to be of significance. In fact the old captive grays are larger than the corresponding albinos, yet they give smaller values for the length of the rows. The smaller body-weights and lengths in the

captive grays up to 60 days is characteristic for the strain, as already pointed out by King (4). There is, then, a positive influence of body-size on the size of the teeth; but, after the period of rapid growth, this becomes less and less significant, and thus age appears as the more important factor.

Associated with body-size are differences in size of teeth according to sex. The females in both strains have a smaller body-size; also from the earliest age, smaller teeth.

In the foregoing presentation many statements touching the relations of the data have been made without presentation of the detailed computations, but the entries in the accompanying tables and charts give the data by which these statements may be tested.

CONCLUSIONS

In the albino, and in the captive gray Norway rats, the molar teeth grow after eruption, both in length and width, with advancing age. This growth involves an extension of the enamel covering the crowns of these teeth. The enamel, therefore, grows in area.

Growth of the molar teeth is most rapid from 37 to about 60 days of age. Within this period, increase in the size of the teeth is positively correlated with increase in the size of the body, but at later ages the influence of body-size becomes insignificant.

The captive grays here studied have teeth somewhat smaller than those of the albinos. In both strains, the teeth of the females are smaller than those of the males of like ages. This relation appears to be correlated with the difference in body-size according to sex.

LITERATURE CITED

(1) Donaldson, Henry H. 1924 The rat: data and reference tables; 2nd ed. The Wistar Institute.

(2) Donaldson, Henry H. 1929 On the growth of the molar teeth after eruption in the albino rat and in the cat. *J. Den. Res. 9*, 285.

(3) Donaldson, Henry H., and French, Harley E. 1927 On the growth in the diameters of the molar teeth of the albino rat, after eruption. *Anat. Rec., 34*, 277.

(4) King, Helen D., and Donaldson, Henry H. 1929 Life processes and size of the body and organs of the gray Norway rat during ten generations in captivity. I. Life processes. II. Size of the body and organs. *Amer. Anat. Memoir*, No. 14. The Wistar Institute.

(5) Wood, Horace E., 2nd and Wood, Florence D. 1931 Refutation of the alleged diametric growth of erupted rat molars. *Anat. Rec., 48*, 169.

SILVER BROMIDE AS A PULP STAIN

A Preliminary Report

J. STAZ, L.D.S., R.C.S. (Eng.)

Department of Histology, University of the Witwatersrand Dental School, Johannesburg, South Africa

While preparing sections in an attempt to demonstrate the innervation of pulp and dentine, it was found that only indifferent results were obtained by using the more common methods of silver and gold reduction. It was therefore decided to employ silver bromide. A saturated solution of this salt in 10 percent ammonium hydroxide was used, as it gives a very fine emulsion with great powers of penetration. It is also highly sensitive to light, which facilitates its reduction. The method follows:

1. Freshly extracted teeth are placed in a 10 percent formalin solution and fixed, the time depending on the size of the teeth.
2. Decalcify.
3. Cut into small portions and place in a 5 percent sodium sulphate solution for 24 hours.
4. Running water, 24 hours.
5. Solution of ammonium hydroxide (5 percent), 24 hours, to remove any traces of decalcifying solution which may be left after (3) and (4); and to remove any impurities in the tap water, which would tend to reduce the silver prematurely. It also impregnates the specimens preparatory to (6).
6. Saturated solution of silver bromide in 10 percent ammonium hydroxide, 24–48 hours. This stage should be protected from light and dust, which would reduce the silver before thorough impregnation has been obtained.
7. The specimens are removed from the ammoniacal solution and exposed to x-rays for 30 seconds. Light acts as a catalyst on the silver bromide, and causes a deeper and more even reduction. The x-rays penetrate into the depth of the specimen, where the precipitation will be fine and evenly distributed. It has been suggested that before exposure to the x-rays the specimens should be soaked for a short period in 0.5 percent or 1 percent

acid solution, to remove the ammonium radicle. This would result in the specimens being thoroughly impregnated with pure silver bromide in a highly light-sensitive state. It has been found in practice, however, that the silver is in an unstable solution, and that the acid in addition to removing the ammonium radicle also dissolves out the silver from the specimens.

8. Without washing, the specimens are placed in hydrokinone or any reducing agent for 24 hours. They should then be black. If they are only a grey or coffee colour, it will be found that after sectioning they are understained.

9. The specimens are then placed in a fixing solution, such as sodium thiosulphate, for 24 hours to prevent washing out during subsequent manipulation.

10. After 24 hours in running water, the sections are dehydrated, cleared, and imbedded in celloidin or paraffin.

11. Thin sections may be cut and mounted without further staining. They may be very lightly counterstained with any stain. Faint staining with haematoxylin and eosin gives a clear and distinct section. (The times given above are approximate, and vary with the size of the tissues.)

In the sections obtained it has been found that the nerve fibres take a black stain, being much darker than the connective-tissue elements, which are also stained by the silver. Certain fibres have been observed entering the dentine from the pulp, which suggests that they may be neurofibrils; further work, however, remains to be done to eliminate the possibility that they are connective-tissue fibres. It is hoped that this work will be the basis of a further report, the present communication being solely concerned with the technique adopted.

HARDNESS STUDY OF TRANSFORMATIONS IN GOLD-COPPER ALLOYS[1]

HARRY H. ASHER, D.D.S., M.S.

Department of Chemistry and Metallurgy, College of Dentistry, University of Illinois, Chicago, Ill.

Since gold and copper form the basic elements of all dental cast-alloys, a study of these two elements was undertaken as part of a program of research on dental alloys, with special reference to possible utilization of the main transformation—cupric-auride formation—in dental practice. It was decided to use hardness analysis followed by microscopic study of the several alloys. It was very heartening, in the midst of this work, to read Kuranow's conclusion (1) that hardness is a more sensitive test than electrical resistance, and that final solution of the problem of transformations in gold-copper alloys depends upon use of hardness and extrusion-pressure methods. After a study of various hardness-testing machines, Shore's Monotron Hardness Indicator was selected for this work. Errors due to variations in individual measurements with this machine were less than those contributed by other experimental factors.

In this research program, the properties of alloys in their cast state are of major interest. Gold and copper therefore, after being alloyed, were to be cast into suitable forms for hardness testing and for microscopic study. It was obviously necessary to start, in all cases, with pure metals, to avoid contaminations by such elements as arsenic or iron in copper, or silver and copper in gold. Because recast alloys are over 2 percent harder then when first made, new alloys, used throughout, were prepared by placing proper proportions of gold and copper in a rose-crucible, which was then heated in a special gas-furnace while a stream of hydrogen was passed into the crucible. The elements were alloyed at 1150°C. for 20 minutes; when, the flame having been removed, hydrogen was allowed to feed into the crucible until the

[1] Presented at the twelfth general meeting of the International Association for Dental Research, Chicago, March 17 and 18, 1934; *J. Den. Res.*, 1934, *14*, 181; June.

alloy cooled to room-temperature. For the casting technic one of the methods proposed by the Bureau of Standards was used because it was standardized and easily duplicated. Wax-patterns were made of Peck's purple-wax, which was warmed and pressed into a steel-mould that measured 20 x 5 x 5 mm. on edge, and which tapered a few degrees from side to side to facilitate ejection of the wax-patterns. This casting technic was selected to include the variables that might occur in dental practice, so that the results might be utilized in further studies. The castings were heated to 600°C. in an electric-furnace and maintained there for 6 hours. The group to be quenched was removed and plunged into cold water; the group to be slowly cooled was allowed to remain, and the furnace cooled at a rate of approximately 1°C. per minute to 450°C. and thereafter at the rate of 0.5°C. per minute to room-temperature. The castings were then marked with a scratch-gauge into 8 equal divisions, and each mark and side was identified by a number and letter. Hardness readings made in this way could be identified with definite areas, and were invaluable aids in the microscopic study.

There is no agreement among workers in this field as to the length of time required for annealing and heat-treating alloys. Kurnakow (1) came to his conclusions regarding proper heat-treatment by finding the period necessary to obtain a constant electrical-resistance curve for an indefinite time. Similarly, degree of equilibrium attained in the alloys in this study was judged from hardness-tests on castings of the same composition but with different heat-treatments. Although no difference was apparent after a 4-hour annealing-period and a 1°C. per-minute cooling-rate, a 6-hour annealing-period and a 1°C. per-minute cooling-rate to 450°C., followed by a 0.5°C. per-minute cooling-rate to room-temperature, were selected to insure complete annealing. Hume-Rothery (2), in a discussion of papers by Haughton and Payne (3) and Kurnakow and Ageew (4), based his arguments for longer annealing treatment on a comparison of their heating and cooling curves outside of the transformation-areas.

The hardness results of this investigation are shown in *fig. 1*. A study of the curves outside of the transformation-regions show that, with slight differences due possibly to experimental error, they are comparable for the annealed and the as-cast conditions. This evidence would indicate that the selected heat-treatment was ample and that a reasonable state of equilibrium had been reached.

A 2.5 mm. tungsten-carbide spherical-impressor was employed throughout the hardness-tests, instead of the 0.75 mm. diamond-spherical-impressor generally used with this machine, because the larger impressor gives

a greater range of readings for soft metals such as alloys of gold and copper. A pre-pressure of 15 kgms. was used to penetrate the surface of castings before readings were taken, since our only interest was the virgin hardness of the cast-alloys. This precaution was taken so that the impressor passed through the surface whose composition and structure may have been affected by oxidation and the subsequent cleaning treatment. The readings, in terms of applied pressure, were taken on each of the marks on the castings. A reading was taken for every 3/200 mm. penetration up to 30/200 mm. penetration, to see that the results lay on a straight increment line. This gave 10 readings for each mark; and, as there were 32 marks on each casting, 320 readings were obtained. In a few cases, readings that were incongruous were disregarded, but no alloy had less than 280 readings. The mean average was calculated and figured for a penetrational depth of 9/200 mm. or 9/5000 inch. This figure then represented the hardness in kgms. for that particular alloy.

The Monotron Scale, however, is not as commonly used as the Diamond Ball Scale or Kilograms Per Square Millimeter Scale. It was found desirable to transpose our readings into one of these scales, and the Kilograms Per Square Millimeter Scale was chosen. At a depth of 9/200 mm. for a 2.5 mm. spherical-impressor, the diameter of the impression is 0.675 mm. The area of this ball-segment is 0.358 sq. mm., the reciprocal of which gives a constant, 2.79, with which the load in kilograms may be multiplied to give a reading of kilograms per sq. mm. These values, with the Monotron readings, are given in *table 1* and are shown graphically in *fig. 1*.

A study of the curve for slowly-cooled alloys shows a similarity to Kurnakow, Zemczuzny, and Zasedatelev's hardness curve (5) for slowly-cooled alloys from 0 percent Au to about 50 percent (*fig. 2*); but from there on there is little similarity. A closer relationship exists between *fig. 1* and Kurnakow and Ageew's diagram of the isothermals of electrical resistance of the gold-copper system (*fig. 3*). Instead of having two maximae, as Kurnakow et al. have at 45 percent Au to 55 percent, *fig. 1* and *table 1* show but one maximum—between 45 and 48 percent. The reason why this maximum does not occur directly on the 50-percent-Au area is probably due to two factors: segregation and cumulative effects of Cu_3Au_2 and CuAu. A discussion of the first phenomenon has been covered in a previous paper (6). Microscopic studies show that, excepting alloys at the extreme ends of the series, segregation takes place, and does not disappear

without an annealing-treatment. Evidences of segregation are seen in many of Kurnakow's photomicrographs (5).

TABLE 1

Readings on Monotron Scale; transposed on Kilograms Per Square Millimeter Scale

ATOMIC PER CENT		WEIGHT PER CENT		HARDNESS IN KILOGRAMS FOR A CONSTANT DEPTH OF 9/200 MM. WITH 2.5-MM. BALL IMPRESSOR		HARDNESS IN KILOGRAMS PER SQUARE MILLIMETER	
Gold	Copper	Gold	Copper	Slowly cooled	Quenched	Slowly cooled	Quenched
100.00	0.00	100.00	0.00	10.506	10.395	29.312	29.002
95.00	5.00	98.33	1.67	14.592	13.908	40.712	39.403
90.00	10.00	96.54	3.46	16.590	16.710	46.286	46.621
85.00	15.00	94.62	5.38	21.165	21.150	59.050	59.109
80.00	20.00	92.56	7.44	24.453	22.497	68.224	62.766
77.50	22.50	91.44	8.56	26.964	25.878	75.230	72.200
75.00	25.00	90.30	9.70	28.866	28.549	82.537	79.642
72.50	27.50	89.09	10.91	29.928	27.906	83.500	77.858
70.00	30.00	87.86	12.14	21.069	27.114	58.782	75.648
65.00	35.00	85.21	14.79	44.346	41.449	123.725	116.643
62.50	37.50	83.11	16.89	60.603	46.092	169.083	128.600
60.00	40.00	82.31	17.69	64.644	46.269	180.457	129.091
57.50	42.50	80.18	19.82	75.740	48.473	211.315	136.077
55.00	45.00	79.13	20.87	84.492	51.339	235.733	143.236
52.50	47.50	76.52	23.48	93.130	56.962	259.833	158.924
50.00	50.00	75.62	24.38	101.748	59.409	283.877	165.711
47.50	52.50	73.73	26.27	107.156	56.615	298.965	157.956
45.00	55.00	71.74	28.26	104.748	56.779	292.247	158.423
42.50	57.50	69.63	30.37	87.891	55.812	245.216	155.715
40.00	60.00	67.40	32.60	65.100	39.600	181.629	110.484
37.50	62.50	65.05	34.95	28.485	28.548	79.473	79.651
35.00	65.00	62.55	37.45	35.850	30.180	100.021	84.202
30.00	70.00	57.07	42.93	32.910	28.650	91.820	79.942
27.50	72.50	54.06	45.94	31.035	28.890	86.588	80.603
25.00	75.00	50.84	49.16	30.282	30.455	84.487	84.670
22.50	77.50	47.33	52.67	30.820	25.230	85.988	70.392
20.00	80.00	43.68	56.32	27.270	26.880	76.083	75.085
15.00	85.00	35.38	64.62	24.870	25.740	69.388	71.815
10.00	90.00	25.63	74.37	24.840	25.260	69.312	70.475
5.00	95.00	14.04	85.96	22.084	25.877	61.614	62.197
0.00	100.00	0.00	100.00	16.243	17.945	45.318	50.067

Fig. 4 shows four photomicrographs of a 25-percent-Au alloy. No single view is representative of this alloy. *D*, the same alloy after

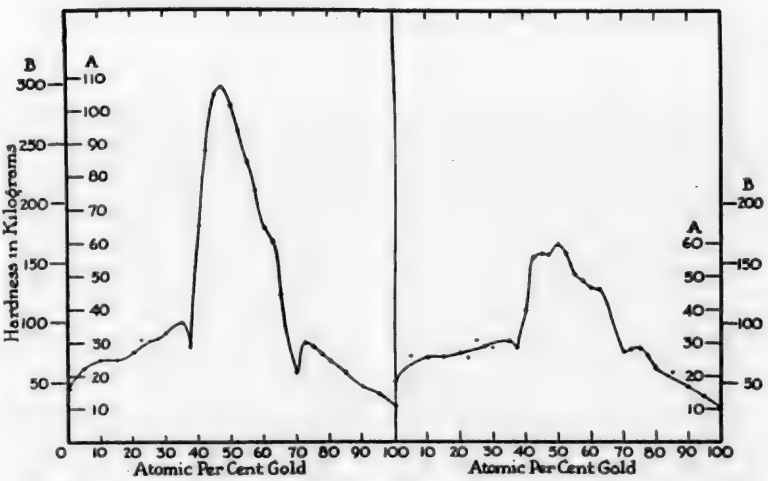

Fig. 1. Author's results for hardness of copper-gold alloys. *Left graph:* slowly cooled. *Right graph:* quenched. *A*, hardness in kilograms for constant depth of 9/200 mm.; 2.5-mm. ball impressor. *B*, hardness in kilograms per sq. mm., for constant diameter of 0.675 mm. (after Brinnell.)

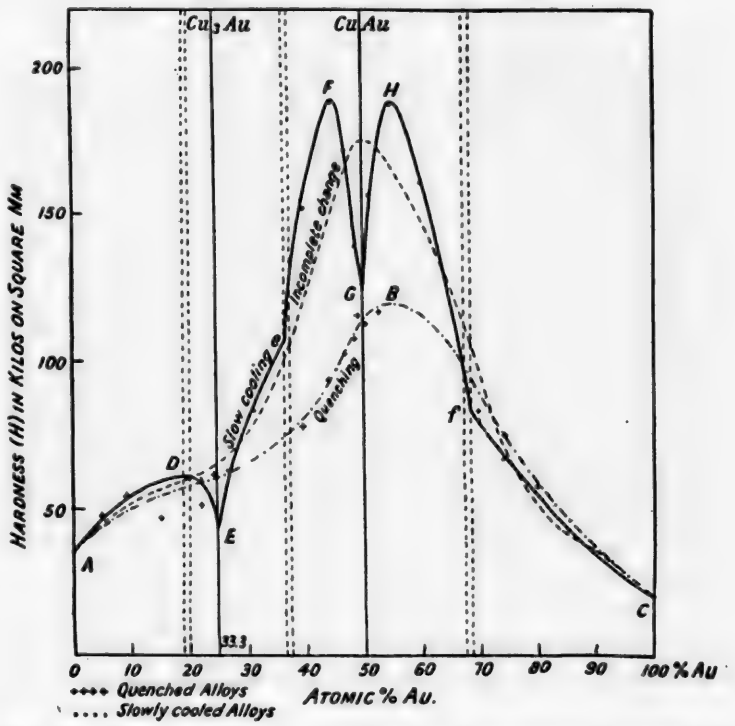

Fig. 2. Graph showing results obtained by Kurnakow, Zemczuzny, and Zasedatelev (5).

annealing, shows complete disappearance of segregation. *Fig. 5* shows two photomicrographs of an 80-percent-Au alloy—*A*, as cast; *B*, annealed. If the alloys were annealed for a greater length of time, probably segregation would be more completely removed and the

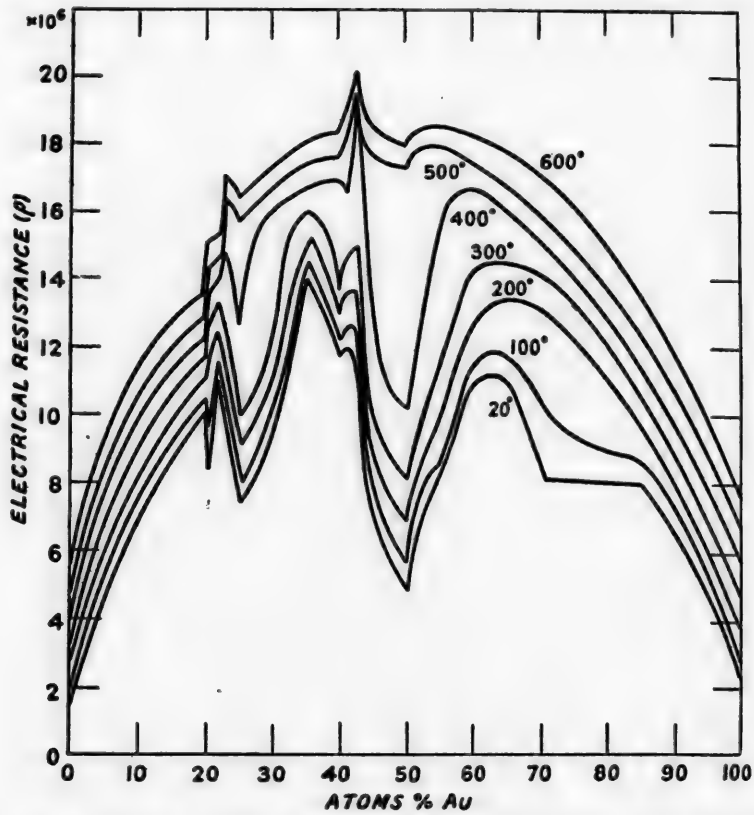

Fig. 3. Diagram of isothermals of electrical resistance of gold-copper system—Kurnakow and Ageew (4).

maximum-hardness point moved closer to the 50-percent area. This matter should be further investigated. A study of Haughton and Payne's equilibrium diagram (*fig. 6*) also shows that maximum hardness, occurring at its particular point, is probably due to cumulative

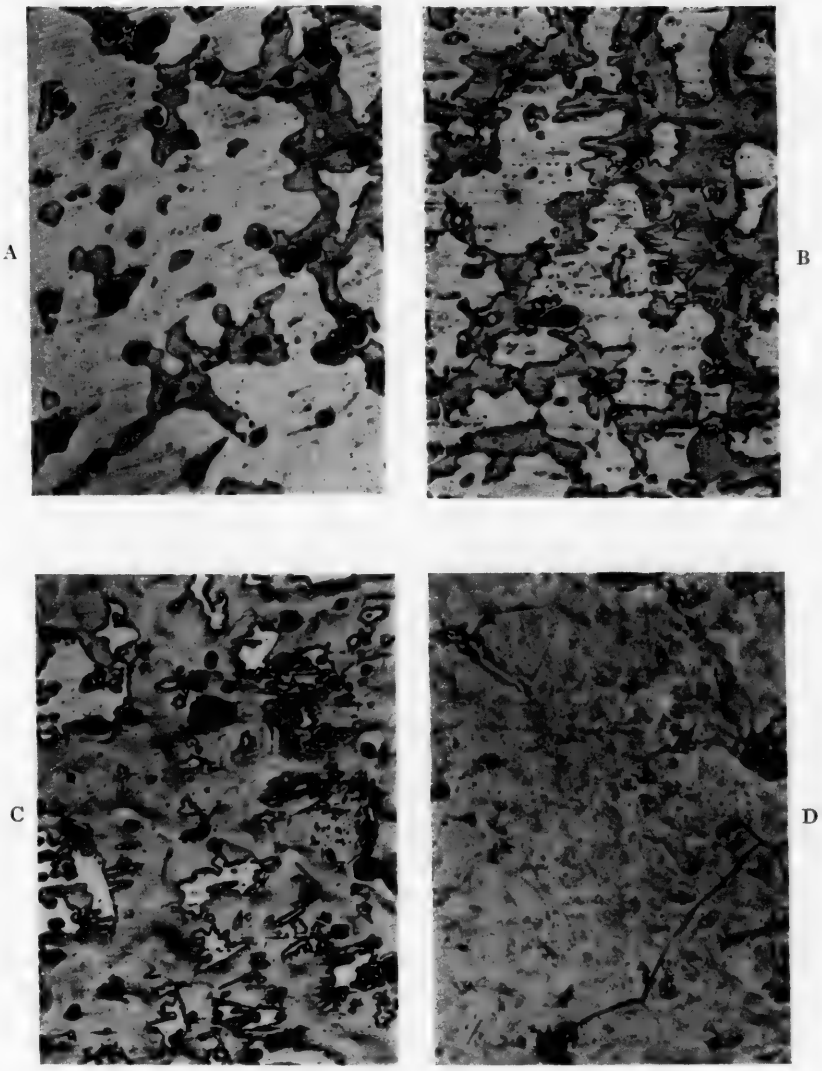

Fig. 4. Photomicrographs of 25-percent-Au alloy. *A*, near top of casting. *B*, near middle. *C*, near sprue-end. *D*, after annealing; showing complete disappearance of segregation. (All ×150.)

effects of CuAu and Cu_3Au_2. By comparing *figs. 1* and *5*, it is apparent that the Cu_3Au formation begins earlier than that at from 22 percent Au to 40 percent, suggested by Kurnakow. The first two show that this formation occurs approximately between 18 and 37 percent Au. It is also interesting to note the close relationship in *figs. 1* and *6* in the gamma region. In both, the part of the phase between 37 and

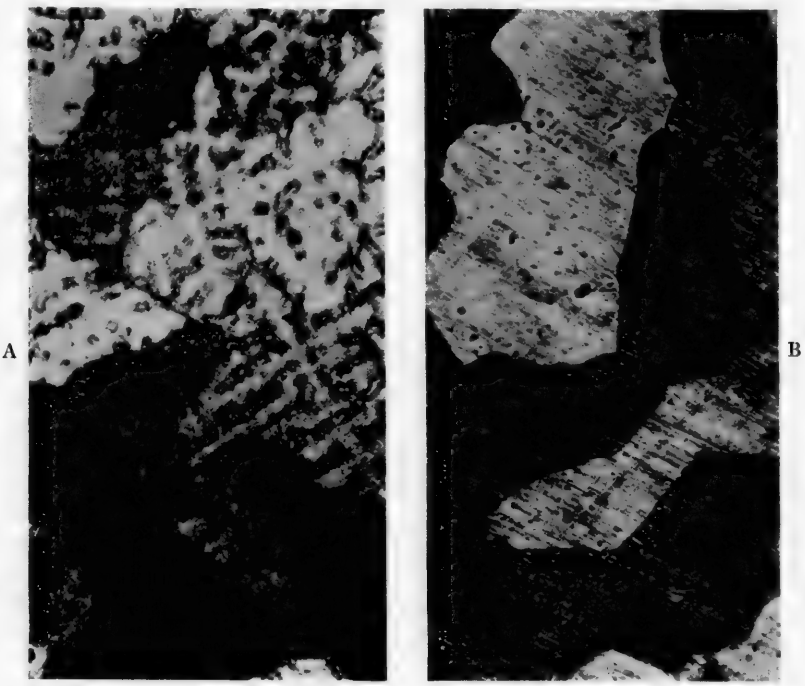

Fig. 5. Photomicrographs of 80-percent-Au alloy. *A*, as cast. *B*, after annealing. (Each ×240.)

50 percent Au is shorter than that between 50 and 70 percent Au, which slopes less acutely from the maximum-hardness point.

A comparison of the curves of the slowly cooled and quenched-alloys (*fig. 1*) shows marked resemblance of all areas with the exception of that between 42.5 and 62.5 percent Au. In the Cu_3Au area, as Merica points out (7), practically no age-hardening occurs owing

to the fact that the lattice of Cu_3Au formation is almost identical with that of solid solution, while that of CuAu formation is vastly different. In the latter case, solid solution has a face-centered cubic lattice, while CuAu formation has a face-centered tetragonal lattice.

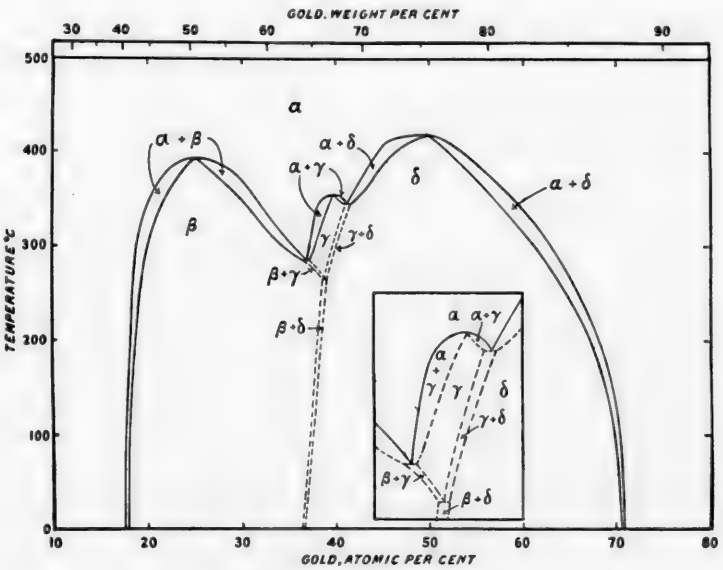

Fig. 6. Equilibrium diagram—Haughton and Payne (3).

Conclusions. Study of the area between 70 and 65 percent Au shows a reaction that may be similar to that of Cu_3Au formation—of a compound $CuAu_3$. Though these may not be similar in nature, still Westgren's opinion (8), that the current explanation of the gold-copper system may not be generally valid, requires attention. From the indications of *fig. 1*, the explanation that the gold-copper system is an unbroken series of solid solutions seems plausible. If it is not correct, then, because of the reaction in the area to the right of CuAu formation (Kurnakow and Ageew's diagram of isothermals), and because the same reaction is definitely similar to that of Cu_3Au formation (*fig. 1*), the presence of another compound of gold and copper may at least be suspected.

However, presence of a definite chemical compound is not proved by the demonstration of a martensitic type of structure as other workers have attempted to show. Even a change in lattice structure in solid solution is not always indicative of formation of a compound. If that occurred, microscopic evidences of it should appear in the neighboring areas, such as are commonly seen in the eutectiferous type of alloys. Microscopic studies were made at ranges up to 500 diameters, in some cases even higher, using regular and dark-field illumination. In none of the sections was there any evidence of martensitic or Widmanstatten type of structure as seen by Kurnakow, et al., and by Haughton and Payne. However, the slowly cooled alloys were not quenched at the various formative periods, as was done by Haughton and Payne, for that was not included in the purpose of this investigation. A further study along this line will be attempted.

Our results appear to lend support to the theory that the gold-copper system is an unbroken series of solid solutions with one major and two minor lattice-changes that are affected in various degrees by heat-treatments.

The author expresses his appreciation to Dr. W. H. Welker for interest, advice, and criticism; to Mr. R. C. Guthrie and Mr. J. A. Comstock for metallographic assistance and criticism; and to Dr. A. F. Shore for aid in translating the results into values in common use.

REFERENCES TO LITERATURE

(1) Kurnakow, N. S., and Ageew, N. W. Physico-chemical study of the gold-copper solid solutions; authors' reply to discussion: *J. Inst. Metals*, 1932, *48*, 312.

(2) Hume-Rothery, W. Discussion of the physico-chemical study of the gold-copper solid solutions: *Ibid.*, 1931, *46*, 502.

(3) Haughton, J. L., and Payne, R. J. N. Transformations in the gold-copper alloys: *Ibid.*, 1931, *46*, 457.

(4) Kurnakow, N. S., and Ageew, N. W. Physico-chemical study of the gold-copper solid solutions: *Ibid.*, 1931, *46*, 481.

(5) Kurnakow, N. S., Zemczuzny, S., and Zasedatelev, M. The transformations in alloys of gold with copper: *Ibid.*, 1916, *15*, 305.

(6) Asher, H. H., and Comstock, J. A. Some causes of variation in hardness of gold-copper castings: *J. Den. Res.*, 1933, *13*, 407.

(7) Merica, P. D. The age-hardening of metals: *Trans. Amer. Inst. Min. and Metal. Engineers*, 1932, *99*, 13.

(8) Westgren, A. Discussion of the physico-chemical study of the gold-copper solid solutions: *J. Inst. Metals*, 1931, *46*, 505.

STUDIES, IN THE RAT, OF SUSCEPTIBILITY TO DENTAL CARIES

II. Effects on Teeth, in Mothers and Young, of Diets Deficient in Calcium and Vitamin D, during Pregnancy, Lactation, and Rearing[1]

THEODOR ROSEBURY, D.D.S., and GENEVIEVE FOLEY, M.A.

Department of Bacteriology, College of Physicians and Surgeons, Columbia University, New York City

In the first report in this series (20), it was noted that enamel hypoplasia occurred in incisors of rats on a low-Ca vitamin-D-free diet, but that apparently only recently formed enamel was affected, molar teeth escaping the deformity. The emphasis placed by Mellanby (15) on possible relationships of enamel defects to dental caries suggested the desirability of attempting to produce defective molar-enamel in rats by dietary means. Since enamel calcification of rat molars is complete at a very early age, it appeared desirable to feed a deficient diet to mothers during pregnancy and lactation, and then to continue the young under the same dietary conditions. Dental effects of this and other conditions, embodying possible factors in caries etiology, form the basis of the present report.

EXPERIMENTAL METHODS

The plan followed in these experiments is represented in *table 1*. The first letter in the "designation" (last column) indicates the mother's diet; the second and third, the diet of the young after weaning.[2] One group of mothers (N) was maintained on an optimal diet to provide a normal standard of growth and tissue structure. Of the groups on the basal test-diet,

[1] Conducted with the aid of a grant from the Commonwealth Fund for research in dental caries. Presented at the tenth general meeting of the International Association for Dental Research, Columbus, March 19–20, 1932: *J. Den. Res.*, 1932, *12*, 462. Received for publication: Oct. 28, 1932.

[2] The rats, all from the Long-Evans hooded strain, were given to us through the courtesy of Dr. P. E. Smith, of the Department of Anatomy.

one (R) received subminimal dosage of vitamin D; another (S), supposedly optimal dosage. Normal adult females were placed on these diets and mated within 9 days. A fourth group (R′), selected from among R animals which had not become pregnant after 26 days, was placed on the basal test-diet without vitamin D. They mated within 10 days, but all litters died within 24 hours after birth. Litters from the other groups were distributed, at weaning, among the nine experimental groups designated in *table 1*.

The *basal test-diet* was a modification of that used previously (20), the Ca:P ratio, although still inadequate, having been improved to provide Ca-

TABLE 1

Arrangement of experimental groups[1]

MOTHERS	YOUNG		
	Diet	Additions	Designations
Stock diet (N)	Stock diet (N)	None	NN, SN, RN
Normal-D, low-Ca (S)	Normal-D, low-Ca (S)	None	NS, SS, RS
		Gum tragacanth	NSt, SSt, RSt
		Glucose ad lib.	NSc, SSc, RSc
		Sodium carbonate	NSx, SSx, RSx
Low-D, low-Ca (R)	Low-D, low-Ca (R)	None	NR, SR, RR
		Gum tragacanth	NRt, SRt, RRt
		Glucose ad lib.	NRc, SRc, RRc
		Sodium carbonate	NRx, SRx, RRx

[1] A fourth group (R′), selected from the R rats that did not become pregnant in 26 days, was placed on "D-free, low Ca" diet. They mated in 10 days, but all litters died within 24 hours after birth.

and vitamin-D deficiencies severe enough to induce tissue changes, yet not too severe to prevent pregnancy, lactation, and rearing of the young for relatively long periods. The two basal diets were prepared as follows (percent): *Modified McCollum* (5) *stock-diet* (*N*): whole-wheat flour (Wheatsworth), 67.5; casein (Merck technical), 15; whole-milk powder (Klim), 10; NaCl, 1; $CaCO_3$, 1.5; Crisco, 3; cod-liver oil (Mead's), 2. *Basal test-diet* (*S*, *R*, *R′*): egg albumin, 20; wheat gluten, 10; Crisco, 15; corn starch, 41; glucose, 10; salt mixture, 4. The *salt mixture* was prepared, and its Ca:P ratio adjusted, as a modification of Osborne-Mendel salt-mixture-IX (17). In its original form, this salt mixture contained no Ca, the PO_4 was reduced three-fourths from the level of the normal IV-salt-mixture, and Na_2CO_3

added equivalent to the remaining PO_4. We modified this by adjusting PO_4 to its original level; adding Ca to the desired ratio; and, to maintain the reaction, adding PO_4 in the form of a neutral buffer-mixture of NaOH and K_2HPO_4. Sufficient Ca was then added, as lactate, to bring the Ca:P ratio to 1:1.5. The P content was 7.3 percent; on the basis of which, 1.5 grams of calcium lactate were added to each 100 grams of basal diet. Analysis of the whole basal diet (S diet, with all additions as below, except spinach) indicated 0.21 percent of Ca and 0.40 percent of P (Ca:P = 1:1.9), of which 0.19 percent of Ca and 0.29 percent of P were contributed by the salt mixture.[3]

In addition, all test diets (excluding stock diet) contained 20 grams of Yeast Vitamin-Harris Powder per kilo; also appropriate dilution of Viosterol in corn oil, 25 grams per kilo. For normal-vitamin-D (S) diets, Viosterol dilution was prepared by dissolving 1.6 grams (Mead's 250D) in 500 grams of corn oil, representing eight times the minimum protective dosage (0.001 percent) recommended for rats by the manufacturers. For R (low-vitamin-D) diets, 0.1 gram of Viosterol was dissolved in 500 grams of corn oil, representing half the minimum protective dosage. For R′ (vitamin-D-free) diet (mothers only), corn oil alone was used. All animals received 5 grams of fresh spinach leaves per day. Each mother on a test diet received wheat-germ oil:[4] 0.2 gram once weekly during pregnancy, and 0.7 gram once weekly during lactation, including the week during which birth was expected. Modifications of litter test-diets represented by "t," "c," and "x" in *table 1* were made as follows: "t"—contained 0.5 percent of powdered gum tragacanth, incorporated with the dry diet, producing an adhesive mixture when moistened; "c"—a separate dish of glucose was kept in the cage with the dish of test diet; "x"—contained 1 percent of Na_2CO_3, to impart an immediate alkaline reaction, intended to increase salivary viscosity. Yeast powder, Viosterol, and corn oil were added to the respective diets freshly every three or four days. All basal diets and water were given *ad lib.*

The animals, kept in cages provided with wide wire-mesh floors, were weighed individually every five days; during the last week of pregnancy the mothers were weighed daily. No attempt was made to feed lactobacilli, but the natural presence of these organisms in mouths of rats, as previously described for animals of a different stock (20)—noted since by other workers (7, 9)—was confirmed in these animals. Data relating to strains we iso-

[3] We are indebted to Drs. Maxwell Karshan and Frances Krasnow for these analytical data.

[4] We are indebted to Dr. Marianne Goettsch for this preparation.

lated are given in another report (19). All litters, weaned and placed on experimental diets 22 days after birth, were carried thereon for 100 days

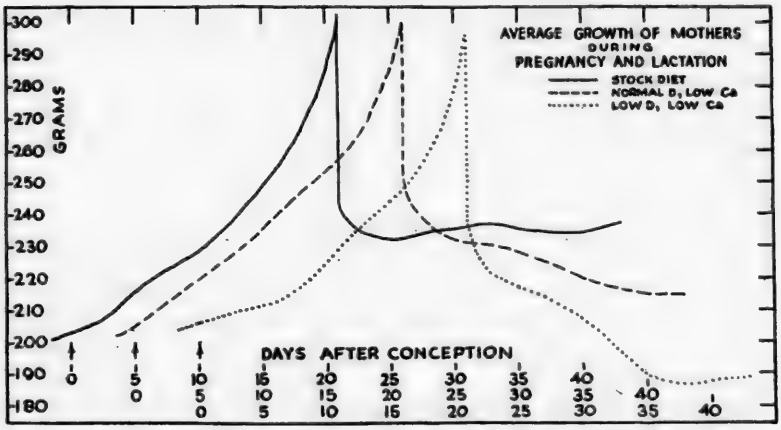

FIG. 1. Graph showing average growth of mothers during pregnancy and lactation. Diets: stock (————); normal in vitamin D, low in Ca (- - - - -); low in vitamin D, low in Ca (. . . . .).

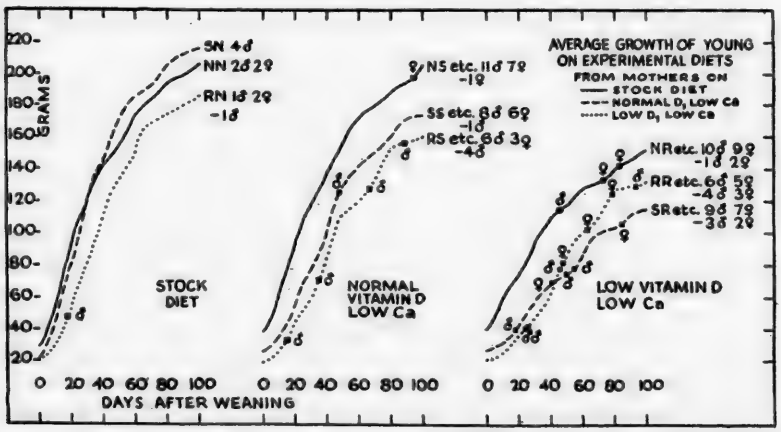

FIG. 2. Graph showing average growth of young on experimental diets. From mothers on stock diet (————); diet normal in vitamin D, low in Ca (- - - - - -); diet low in vitamin D, low in Ca (. . . . .).

after weaning. Some mothers were killed immediately after weaning litters; others were permitted to continue, without changing dietary condi-

tions, for about 100 days. Celloidin sections, stained with hematoxylin and eosin, were prepared from decalcified mandibles and tibias of all animals. Ground sections, some by the collololith method, stained with hematoxylin and eosin—others unembedded and unstained—were prepared from mandibles of about half the litter animals, including all in group R .

RESULTS

GROWTH. Curves for average growth of *mothers* (*fig. 1*) indicate little difference among the three diet-groups during pregnancy. During lactation, however, although stock-diet animals maintained their weight, those on normal-vitamin-D low-Ca diet (S) lost weight steadily; those on low-vitamin-D low-Ca diet (R) averaged nearly

TABLE 2

Average data relating to pregnancy and lactation

DIET	MOTHERS					YOUNG				
	No.	At conception		Gain during preg'y, gms.	Loss during lact'n, gms.	No. at birth	Deaths before weaning, gms.	Litter weights at weaning		
		Age, days	Weight, gms.					Limits, gms.	Av., gms.	Total, gms.
N	5	122.8	203.8	99.0	65.4	9.6	1.4	22–44	32.6	271.2
S	6	119.8	205.3	94.2	84.7	9.3	1.2	13–40	23.9	198.3
R	4	126.5	206.3	89.3	106.0	8.5	2.3[1]	12–28	18.7	144.3
R′	3	157.0	226.3	55.3	.....	8.7	8.7	.....	....	.....

[1] One litter of 8 was destroyed by the mother between the 20th and the 22nd day after birth. Litter weights are therefore averaged on the basis of three mothers.

20 grams less at the end of lactation than at the beginning of pregnancy. The young evidently drew more heavily on the mother's resources during lactation than during pregnancy, probably, as Macomber (14) has pointed out, because active calcification begins postnatally in the rat. Averages are given in *table 2*, where it may also be noted that litter size did not vary significantly. In the R group, one litter of 8 was destroyed by the mother during the last days of lactation. If this may be discounted, the mortality of offspring during lactation did not differ significantly among the groups receiving vitamin D. On the other hand, in the R′ group deprived of vitamin D, the litters, which did not differ from normal in number or (apparently) size of individuals, all died within 24 hours after birth. The most striking gross defect in the young, produced by deficient diets

up to the time of weaning, was reduction in individual weights. The similarity of the weights for S and R groups indicates that deficiency of Ca, rather than of vitamin D, was the conditioning factor in this reduction. The curves for average growth of the *young* (*fig. 2*) are grouped without regard to "t", "c", or "x" subgroups, which were not distinctive, excepting that those in "c" (separate glucose) were generally poorest. The litters from stock-diet (N) mothers, on stock and on normal-vitamin-D (S) diets—and those from normal-vitamin-D-diet mothers on stock diet (NN, NS, and SN)—may be considered as varying within normal limits. The other curves are all relatively subnormal. It is noteworthy that the normal-vitamin-D low-Ca diet, when carried through two generations (SS), was less than adequate. That this inadequacy was correlated with dietary Ca-deficiency is attested by histological findings to be described. The various R diets, although subnormal, were not markedly so. *Mortalities* are represented by points (and sex signs) on the curves in *fig. 2*, each representing death at the indicated time. The number and position of these points are evidence that the mortalities were directly caused by dietary conditions. Deaths occurred early in groups transferred at weaning to an improved diet, and vice versa.

HISTOLOGICAL FINDINGS. Lesions on abraded cusps of molars, which may correspond to that described by Klein and Shelling (10) as experimental dental caries, were found in ten instances—three mothers (2S, 1R) and seven young (1N, 1NSt, 2NSc, 1SSt, 1SSc, 1NRx). An example of this lesion is shown in *fig. 3*. Otherwise no dental caries was observed, although evidence of impaction of food in molar fissures was plentiful. Mothers showing this lesion had been on experimental diets for 156, 46, and 122 days, respectively, while all the young survived the 100-day experimental period. In each case, the lesion was a beginning disintegrative change in dentin at the abraded summit; gross cavitation had not occurred. It is apparent, from the distribution of this lesion, that it cannot be regarded as arising from any of the experimental conditions under control. This matter is considered further below.

Data relating to incidence of pathological changes in teeth and bones of the young are presented in *table 3*; the changes are described below, where supplementary data relating to mothers and miscellaneous

TABLE 3

Data relating to pathological changes in teeth and bone of young rats on experimental diets

GROUP		DEATHS				MOLARS								INCISORS								ALV. BONE			EPIPHYSEAL CARTILAGE				
Symbol	No.	No.	Days	AV. GAIN PER DAY, GMS.	NORMAL THROUGHOUT	Normal	Loc. dentinoid-odontoblast irregularity: Mild	Loc. dentinoid-odontoblast irregularity: Severe	Uniform dentinoid	Pulp stroma-cell change: Mild	Pulp stroma-cell change: Moderate	Death pulp change	Enamel irregular	Normal	Dentinoid mottling: Mild	Dentinoid mottling: Severe	Dentinoid mottling: Extreme	Enamel hypoplasia: Post.	Enamel hypoplasia: Unif.	Pulp stroma change	Death: pulp change	Normal	Defective	V. defective	Data for	Normal	Irregular	Broad	Broad-irreg.
NN	4			2.00	4	4								4								4			4	4			
SN	4			1.92	3	4								3	1							4			4	4			
RN	3	1	19	1.34	2	2						1*		2							1*	3			—				
N	11	1		1.71	9	10						1*		9	1						1*	11			8	8			
NS	5			1.53	2	2	3			3				2	3			1		2		5			2	1	1		
NSt	4			1.72	2	2	2			1				4								4			3	3			
NSc	5			1.72	1	1	4			1				1	4			1				5			2	2			
NSx	4	1	94	1.56	1	2	2			2				3	1d			1d		1d		3	1d		2	1	1		
NS, etc.	18	1		1.63	6	7	11			7				10	8			3		3		17	1d		9	7	2		
SS	3	1	48	1.87	1	2	1			2				2		1d				1d		2	1d		2	2			
SSt	4			1.39		1	3			2				3	1							4			3	2	1		
SSc	4			1.19			3	1		2					4					1		4			4	2	2		
SSx	3			1.53	1	1	2			1				2						1		3			1		1		
SS, etc.	14	1		1.46	2	4	9	1		7				7	5	1d				3		13	1d		10	6	4		
RS	2	1	15	0.93			1		1d	1		1		1							1	1	1		1	1			
RSt	2			1.51			1	1		1				1	1							2			—				
RSc	3	1	67	1.19			1	2		1		1	1d	1	2						1	2	1d		1	1			
RSx	1	1	89	1.62			1d					1	1d		1d			1d			1	1d			1			1d	
RS, etc.	8	3†		1.26			4	3	1d	3		3	2d	3	4			1d			3	6	2		3	2		1d	
NR	4			1.34			2		4	3						4		3					4		3		1	2	
NRt	5	1	73	1.10					5	1	2	1				5		5			1		5		3		1	2	
NRc	5	2	46, 83	0.95				1	4	1	2*	2				5		4		2*	2		4	1	3			2	1
NRx	5			1.39				2	3	1						5		2				1	4		3		3		
NR, etc.	19	3		1.12			2	3	16	6	4	3				19		14		2*	3	1	17	1	12		5	6	1
SR	5	1	19	0.89					5	2		1	1			5		3		1	1		5		3		3		
SRt	3			1.20					3	1	1					3		3					3		3		1	1	1
SRc	4	3	85, 55, 50	0.61					4			1	1			4		2	1d		1		4		4		1	1	3
SRx	4	1	41	0.75					4	3		1				4		4			1		3	1	4	1	2	1	
SR, etc.	16	5		0.73					16	6	1	3	2			16		12	1d	1	3		15	1	14	1	3	6	4
RR	3	2	94, 79	0.86					3		1	2				3		1	2d	1	2		3		2			1	1
RRt	3	3	63, 48, 24	0.98					3d			3*				1d	2d	2	3d		3		3d		2d			2d	
RRc	2	1	45	0.83					2	1		1	1d			2		2			1		2		—				
RRx	3	1	25	0.90					3	1	1	1	1			3		1	2	1	1		3		3	1	1	1	
RR, etc.	11	7		0.89					11	2	2	7	2			9	2d	4	7	2	7		11		7	1	1	4	1

* Atypical in one case. d = Animal that died. † Does not include one animal about which no data were available.

additional animals are given separately. The characteristics accepted as normal, based on the histology of stock-diet controls, were the following (*figs. 4, 8, 14*), pathological changes having been estimated by comparison with this normal picture: uncalcified enamel, and ameloblasts in the posterior portion of incisors (decalcified sections), were very regular. Calcified enamel in incisors was regular; in molars generally somewhat undulant or pitted in outline, making detection of slight hypoplasias difficult by comparison. Dentinoid was generally absent in molars, appearing locally as narrow strips in isolated regions, and narrow and regular in incisors. Contour irregularities of pulpal walls were common in molar roots, accompanied by corresponding irregularities of odontoblasts. In incisors, odontoblasts were consistently regular in arrangement, but in molar pulp-chambers regularity was rare. In molars, stroma cells of pulps were generally elongated, varying from fusiform to stellate; their disposition suggested crowding due to pressure of encroaching dentin walls. Relatively rapid growth of these teeth may account for this circumstance, and for apparent hyperemia in adult pulp, since, in young pulp (page 374), these characteristics were not evident. In incisors, stroma cells did not appear compressed, and nuclei were rounder than those in molar pulps, although still definitely angular in outline. Alveolar bone was uniformly stained; epiphyseal cartilages of tibias were narrow and regular.

Changes in enamel: the first observed change occurred in uncalcified enamel in posterior portions of incisors, and consisted of local hypoplastic areas, below which ameloblasts were separated and irregular (*fig. 5*). This change has been reported by Klein (8). On the more deficient diets, similar changes occurred anteriorly in calcified enamel. In extreme instances, incisal enamel was reduced posteriorly to narrow irregular amorphous bands (*fig. 6*), ameloblasts not being visible, and was hypoplastic throughout (*fig. 7*). While slight contour changes in incisal enamel were obvious by comparison with perfect normal regularity, determination of such changes in molars was difficult, because irregularity occurred even in normals. No obvious hypoplasia of molar enamel was observed; but in general, irregularities of contour, and apparent reduction in breadth at contact surfaces, were more common and pronounced in the more deficient diet-groups. It is

probable, therefore, particularly in view of the extreme hypoplasia seen in incisal enamel, that some degree of defect was produced in molar enamel.

In *dentin*, the earliest change was formation of apparently uncalcified dentinoid in molars or incisors, or both. In molars, on the less deficient diets, this change consisted of irregularity and local broadening of dentinoid (*fig. 10*), chiefly in roots and on pulpal floors. In a few instances root irregularity was marked (*fig. 13*). On the more deficient diets, irregularity was reduced or absent, the dentinoid being generally broad (*fig. 9*). This change was described in a previous report (20), and by others (3, 12, 16). In incisors, no dentinoid irregularity was noted, but broadening occurred, accompanied in severe cases by marked globulation of the calcified dentin (*fig. 5*). In the severest cases, dentin was reduced to a narrow band of nearly or totally uncalcified tissue (*fig. 6*).

Changes in pulps, of both molars and incisors, were slight at most, excepting animals that died before the conclusion of the experiment. On the less deficient diets, molar odontoblasts were deformed, chiefly in the roots, in areas where dentinoid was irregular (*figs. 11, 12*). Such irregularity was largely absent from the more deficient diet-groups, where dentinoid formation was general and uniform rather than localized and irregular. In some instances this change appeared to result from deformation or folding of unevenly softened teeth, producing alteration of odontoblasts in deformed areas (*fig. 12*). Where dentinoid occurred uniformly, even though the uncalcified zone was extensive, deformation was absent. No corresponding irregularity occurred in incisor pulps. Here, in animals that survived the experiment, odontoblasts appeared normal even when dentin and enamel were markedly altered (*fig. 16*). *Stroma cells* in molar pulps of most animals that survived the experiment showed slight local or general alterations—enlargement of nuclei, loss of angularity, and irregularities of staining (*fig. 18*). These changes, which may represent slight edema, occurred more extensively in the third molar than in the other two; in most cases areas of apparently normal pulp-stroma were present. Some animals, despite extensive calcification defects, showed no appreciable pulp abnormality (*fig. 15*). Similar change in stroma cells was present in some incisor pulps, but even less markedly

than in molars. In most instances no abnormality was observable in incisor pulps, although other tissue changes were extensive (*fig. 16*). Characteristic and severe atrophic change occurred in pulps of molars and incisors of most animals that *did not survive* the experiment (*fig. 17*). Animals killed after a 100-day experimental period did not show this change; therefore, it might have been produced post-mortem.

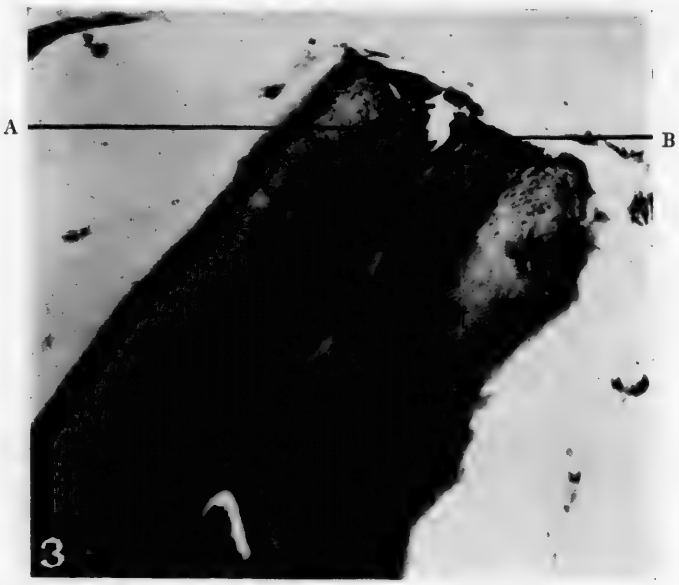

FIG. 3 (14 SSt × 200). Beginning disintegration of summit of cusp of first molar. Lesion (*A*) occurs at center of worn dentin surface (*B*), not normally covered with enamel. Diet: normal-vitamin-D, low-Ca. From mother on same diet. Killed 100 days after weaning.

Incisal odontoblasts appeared to be reduced in lateral diameters, and nuclei were shrunken and rounded. In molars, presumably the same change appeared, generally as an irregular broad zone of small round nuclei. Odontoblasts were absent in some instances, and separated from the dentin wall in others, or replaced by a zone of fibrosis. Stroma-cell nuclei of incisors and molars were similarly reduced in size and rounded, and separated by granular basis-substance. In

FIG. 4 (4 SN × 100). Normal lower incisor, lower wall of forming tooth, posteriorly. Ameloblasts (*A*), enamel matrix (*B*), and odontoblasts (*C*), regularly aligned; dentinoid (*D*) narrow and even. Stock diet; from mother on normal-vitamin-D low-Ca diet. Killed 100 days after weaning.

FIG. 5 (27 SRt × 200). Lower incisor, lower wall of forming tooth, posteriorly. Broadened dentinoid at *A*, globulation of dentin at *B*, hypoplastic enamel at *C*, distorted ameloblasts at *D*. Low-vitamin-D low-Ca diet; from mother on normal-vitamin-D low-Ca diet. Killed 100 days after weaning.

FIG. 6 (95 RRt × 160). Lower incisor, lower wall of forming tooth, posteriorly; shows extreme changes. Dentin (*A*) narrow; nearly or totally uncalcified. Enamel matrix (*B*) represented by narrow band of amorphous material; ameloblasts not in evidence. *C*, odontoblasts. *D*, pulp. Low-vitamin-D low-Ca diet; from mother on same diet. Died 48 days after weaning.

FIG. 7 (99 RRx × 25). Ground section, unstained. Tip of lower incisor, shows enamel hypoplasia (*A*). *B*, dentin. Low-vitamin-D low-Ca diet; from mother on same diet. Killed 100 days after weaning.

FIG. 8 (145 NSt × 25). Normal lower molars, showing characteristics of normal dentin (*A*). Note dentinoid (*B*): narrow in some regions, not apparent in others. Normal-vitamin-D low-Ca diet; from mother on stock diet. Killed 100 days after weaning.

FIG. 9 (95 RRt × 20). Lower molars, showing generalized broad dentinoid (*A*), without deformation of roots. Low-vitamin-D low-Ca diet; from mother on same diet. Died 48 days after weaning.

FIG. 10 (153 NSc × 20). Lower molars, showing local broadening of dentinoid of roots (*A*) and pulpal floor (*B*). Normal-vitamin-D low-Ca diet; from mother on stock diet. Killed 100 days after weaning.

FIG. 11 (26 SSc × 120). Lower first-molar root. *A*, dentinoid. *B*, irregularity of dentinoid and odontoblasts. *C*, pulp. Normal-vitamin-D low-Ca diet; from mother on same diet. Killed 100 days after weaning.

FIG. 12 (18 SRx × 240). Pulpal floor of lower first molar. *A*, pulp. *B*, dentinoid. *C*, tuft of capillaries enclosed by dentinoid, apparently as result of folding of softened tooth. Note mass of deformed dentinoid above. Low-vitamin-D low-Ca diet; from mother on normal-vitamin-D low-Ca diet. Killed 100 days after weaning.

FIG. 13 (26 SSc × 25). Lower third molar, showing severe deformation of roots. Normal-vitamin-D low-Ca diet; from mother on same diet. Killed 100 days after weaning.

FIG. 14 (65 NN × 320). Normal molar pulp. *A*, dentin. *B*, dentinoid. Odontoblasts (*C*) irregularly disposed. Stroma cells (*D*), generally elongated, and apparently compressed. Stock diet; from mother on same diet. Killed 100 days after weaning.

FIG. 15 (46 NRx × 320). Molar pulp, showing apparently normal structures, despite excessive dentinoid formation. *A*, pulp. *B*, odontoblasts. *C*, dentinoid. Low-vitamin-D low-Ca diet; from mother on stock diet. Killed 100 days after weaning.

FIG. 16 (110 RRx × 320). Part of lower wall of lower incisor, posteriorly, showing normal pulp despite severe dentinoid formation. *A*, pulp. *B*, odontoblasts. *C*, dentinoid. *D*, calcified dentin. Low-vitamin-D low-Ca diet; from mother on same diet. Killed 100 days after weaning.

FIG. 17 (88 RR × 320). Molar pulp, showing alterations correlated with death of animal during experimental period; hemorrhage (*D*). *A*, dentinoid. *B*, odontoblast layer. *C*, pulp. Low-vitamin-D low-Ca diet, from mother on same diet. Died 94 days after weaning.

FIG. 18 (93 RRx × 320). Molar pulp. *A*, dentinoid. *B*, odontoblasts, showing little if any abnormality. *C*, pulp regionally normal. *D*, area of pulp showing enlargement of stroma-cell nuclei. Low-vitamin-D low-Ca diet; from mother on same diet. Killed 100 days after weaning.

some pulps, normal vascularity was reduced; in most cases, however, notably increased, and extensive hemorrhage was present.

Changes in alveolar bone, similar to those previously reported (20), consisted of formation of apparently uncalcified osteoid bordering the bony trabeculae. In tibias, changes were generally slight, as would be expected with low-Ca diets. On the less deficient diets, epiphyseal cartilage was irregular without being appreciably broader than normal, while different degrees of broadening, with or without irregularity, occurred on the more deficient diets.

FIG. 19 (136 × 120). Cusps of unerupted molars, showing discontinuity of ameloblasts (*A*); indicating dentin at cusp summit (*B*), normally exposed before wear begins. *C*, ameloblasts. *D*, oral epithelium. Stock diet. Killed 19 days after birth.

Data relating to mothers. Shorter-term mothers in each group were killed at weaning of litters. Others continued for 100 days thereafter. Information regarding calcified enamel was lacking, owing to absence of ground sections.

N: stock diet; five animals, 62, 68, 68, 148, and 167 days. Normal as described above, except that dentinoid was slightly broad in incisor, in one case.

S: normal-vitamin-D low-Ca diet; six animals, 46, 46, 46, 48, 153, and 156 days. Slight disturbances of calcification in incisors, involving dentin only; otherwise all tissues normal.

R: low-vitamin-D low-Ca diet; four animals, 50, 61, 125, and 131 days. Last destroyed her litter toward end of lactation period. Moderately severe changes in bone

and incisors, including both dentin and enamel; latter in shorter-term animals only. In some cases dentinoid formation occurred in incisor posteriorly only; in others, throughout length of tooth; difference not correlated with length of experimental period. Molars showed slight irregularities of dentinoid only. Tibias, normal. In two animals (61 and 131 days), pulps normal throughout; in one (125 days), only pulp abnormality was slight stroma nuclear enlargement in incisors; in other (50 days), condition more marked, and present locally in molars.

R′: vitamin-D-free low-Ca diet; three animals, 31, 126, and 128 days; each plus 26 days previously on R diet. None lactated; showed changes similar to R group, *less* severely. Shorter-term animal normal but for slight broadening of incisal dentinoid; others, moderate changes in incisal dentin, slight irregularities in incisal enamel, and slight bone changes. One (128 days) showed dentinoid irregularity in molars. Tibias, no abnormality. In shorter-term animal, moderate enlargement of incisal pulp stroma-nuclei; one of others (126 days) showed this change in molars and incisors; other had normal pulps.

Data relating to miscellaneous animals. R—19 days: one of poorer members of litter of mother on R diet; killed 19 days after birth. Head and extremities disproportionately large, and body stunted. Jaws and tibia compared with those of stock-diet animals, same age. In R animal, molars had not erupted. Dentin poorly calcified, but odontoblasts and pulps, and enamel and ameloblasts, normal. Tibial shaft calcified, but epiphysis showed only beginning cellular concentration. In stock animal, first molar just erupted. In unerupted teeth, layers of enamel organ terminated occlusally at side of dentin cusp, indicating cusps never completely covered with enamel (*fig. 19*). Dental tissues normal. Molar pulp-chambers very large; neither compression of stroma cells, nor apparent hyperemia, characteristic of adult pulps. Alveolar bone normal. Tibia: calcification of epiphysis well advanced, leaving broad regular band of epiphyseal cartilage.

R′-young at birth; six animals, two from each mother on R′ diet; dead at or soon after birth; jaws and tibias compared with those of stock-diet animal killed at birth. Normal animal: germs of first and second molars and incisor present, formation of enamel and dentin beginning, alveolar bone present, ossification of tibial shaft advanced, no epiphyseal ossification. R′ animals: ameloblasts and odontoblasts met without tissue formation; no apparent retardation of development; molar germs and incisors in about same stages as normal; dentin papillae normal; alveolar bone present, and tibial ossification as well forward, or nearly so; incisal dentin, and alveolar and tibial cancellated bone, perhaps poorer in quality.

DISCUSSION

Consideration of the pathological data in general, in relation to dental effects of the dietary conditions studied, with both supposedly adequate and reduced vitamin D, indicates that vitamin D (in the dosage employed) does not fully protect the teeth of either mothers or young when the Ca:P ratio is inadequate. Dental disturbances on normal-vitamin-D (S) diets were different, in kind as well as degree, from those on the more deficient diets. In the former, dentinoid was local and irregular; in the latter, general and more uniform. If the

difference in effects between low-vitamin-D (R), and normal-vitamin-D (S), diets was one of degree of severity only, then dentinoid irregularity in the latter group was not a reflection of odontoblastic disturbance, since these cells were not generally affected in R groups. Local dentinoid formation on S diets might have arisen from slight local variation in the nutrition of dentin-forming cells. Distortion of these areas might then have resulted from inequality of surrounding pressures, which in turn produced observed morphological changes in odontoblasts.

Total mortality of young from mothers on R′ diet, as compared with results on R diet, suggests that vitamin-D dosage in the latter approximated limiting conditions for survival of young on the basal diet. These two rations differed only in that one (R′) contained no vitamin D; the other (R), 0.0005 percent of 250D Viosterol, estimated as half the minimum dosage. The two groups also differed, however, in that R′ mothers had been kept on R diet for 26 days before mating, and thus perhaps suffered partial depletion of vitamin stores. Grant (6) found that when Ca is deficient, higher vitamin-D dosage of mothers is necessary to insure normal bone-growth in the young. Yet since vitamin-D requirements of non-pregnant adults are probably well below those of growing young, the R diet might have contained vitamin D sufficient for resting adults.

The data indicate that length of exposure of the animal to deficient diets, rather than period of life during which the deficiency operated, was the more potent factor in retarding growth, damaging the dental apparatus, and inducing mortality. Thus, young that died at birth from mothers on R′ diet were very nearly normal histologically. The R animal killed 19 days after birth, although exceptionally defective in gross appearance, histologically showed only moderate retardation of development and calcification. On the other hand, animals from stock-diet mothers, transferred at weaning to R diet, all suffered well-marked alterations of dental tissues, while young from R mothers transferred to stock diet were unaffected after 100 days. The effects of deficient diet in early life were thus not permanent, but overcome during periods of adequate nutrition.

The significance of the described pulpal changes in these animals is doubtful. Bracco (3) described pulp changes in rats on low-Ca diets,

and Blackberg and Berke (2) reported severe inflammatory changes. Our own findings, more in accord with those of Downs (4), suggest that at most effects on pulps are slight. Our evidence of their absence, in instances of severe alteration of hard dental tissues, seems clearly to indicate that defects in calcification do not depend upon previous morphological change within the pulp. The correlation of death of the animal with more severe pulp-changes described by Blackberg and Berke (2) may account for the effect reported. Since this change was not shown in any instance by animals killed in our experiments 100 days after weaning, even though these animals lived under the same dietary conditions for a longer period, it is possible the effect was not due directly to dietary deficiency.

The disintegrative molar-cusp lesion in a few of these animals was noted in a previous publication (18), in which it was pointed out as well that important differences exist between this lesion and typical dental caries in human beings—and in rats, as shown by Shibata (22) and Barker (1). Since this investigation was completed, further studies of dental caries in this laboratory have confirmed this conclusion—and the work of Shibata—by establishing the fact that caries of molar-enamel fissures, closely simulating dental caries in young human beings, may be produced in young rats, well within the experimental period of the present research. A preliminary report of this work has been published (21).[5] These findings show that the rat is susceptible to true dental caries, which may be produced at will in fissures of its molars during the animal's active growth. Accordingly, negative findings in this regard in the present study assume added value. But until evidence relating to etiology of this lesion has been accumulated sufficiently to warrant definite conclusions, discussion of this matter will be deferred. However, it is clear from this report, and the preceding one in this series (20), that the observations of Klein and McCollum (11), to the effect that caries incidence is not necessarily related to defective tooth-structure, are fully justified. To this we may add the further note, based on the presumptive evidence given, that enamel of susceptible teeth may be defective without supervention of caries.

[5] A complete report of this work (III in this series) was published, since the receipt of the present paper, by Rosebury, Karshan, and Foley: *J. Den. Res.*, 1933, *13*, 379.—[*Ed.*]

SUMMARY AND CONCLUSIONS

Eighteen normal adult females were divided into four groups, one of which received diet low in Ca and vitamin D; the others, comparable vitamin-D-free and adequate-vitamin-D diets, and a modified McCollum-stock diet. These were mated to normal males, carried through pregnancy and lactation, and in some instances for 100 days thereafter, under the same dietary conditions. Litters, at weaning, were placed correspondingly on the same diets; or on modifications involving additions to increase adhesiveness, and to provide excess of fermentable carbohydrate. The young that survived were carried under these conditions for 100 days after weaning. Histological analysis of mandibles of both mothers and young was made in all cases. The results for 125 animals may be summarized as follows:

1. Diets having a Ca:P ratio of 1:1.9, with or without deficiency of vitamin D, given continuously through prenatal life and for 122 days thereafter, did not induce caries. Supplementing these diets with excess of fermentable carbohydrate, and with agents promoting adhesiveness, likewise did not induce caries. Mothers which bore litters on these diets, and were subsequently carried on them through lactation and for three months thereafter, were also free from caries. Incipient destructive molar-cusp lesions, distinct from dental caries, which occurred in a few cases among the experimental groups, were apparently not dependent upon any experimental conditions under control.

2. The deficient Ca:P ratio employed, even with adequate vitamin-D, induced slightly subnormal growth when continued into the second generation. With partial deficiency of vitamin D, this diet induced marked loss of weight in mothers during lactation, and subnormal growth of offspring, whether of the second generation on this diet, or from mothers on more adequate diets. Young from mothers bred on this diet, without any vitamin D, all died within 24 hours after birth.

3. With partial deficiency of vitamin D, the deficient Ca:P ratio resulted in formation of defectively calcified teeth and bone in the young. In mothers, incisors were affected more than molars. Addition of adequate vitamin-D to this diet did not prevent defective calcification of teeth, although it resulted in general in formation of normal bone.

4. Changes in pulps of offspring on various deficient diets were slight—excluding animals that died before the end of the experiment, and were not essential antecedents of changes in the hard tissues. Instances are cited of defectively calcified teeth having pulps that appeared to be normal.

5. The findings include evidence that cuspal dentin of molar teeth in rats is not completely covered by enamel, even before eruption.

REFERENCES TO LITERATURE

(1) Barker, F. J. 1931 *J. Amer. Den. Assoc., 18,* 17.
(2) Blackberg, S. N., and Berke, J. D. 1932 *J. Den. Res., 12,* 349.
(3) Bracco, J. J. 1923 *Compt. Rend. Soc. de Biol., 89,* 453.
(4) Downs, W. G. 1932 *J. Den. Res., 12,* 363.
(5) Evans, H. M., and Bishop, K. S. 1922 *J. Metab. Res., 1,* 319.
(6) Grant, A. H. 1926 *Amer. J. Hyg., 6,* 228.
(7) Hoppert, C. A., Webber, P. A., and Canniff, T. L. 1932 *J. Den. Res., 12,* 161.
(8) Klein, H. 1931 *J. Amer. Den. Assoc., 18,* 866. (9) With Harris, M. H. 1931 *J. Den. Res., 11,* 149. (10) With Shelling, D. H. 1931 *Ibid., 11,* 151. (11) With McCollum, E. V. 1932 *Ibid., 12,* 524.
(12) Kronfeld, R., and Barker, F. J. 1932 *J. Amer. Den. Assoc., 19,* 105.
(13) Lilly, C. A. 1932 *J. Nutrition, 5,* 175.
(14) Macomber, D. 1927 *J. Amer. Med. Assoc., 88,* 6.
(15) Mellanby, M. 1928 *Physiol. Rev., 8,* 545.
(16) Orban, B. 1927 *J. Amer. Den. Assoc., 14,* 1619.
(17) Osborne, T. B., and Mendel, L. B. 1918 *J. Biol. Chem., 34,* 131.
(18) Rosebury, T. 1932 *J. Den. Res., 12,* 255. (19) 1932 *J. Bacteriology, 24,* 321. (20) With Karshan, M. 1931 *J. Den. Res., 11,* 121, 137. (21) With Karshan, M., and Foley, G. 1932 *Ibid., 12,* 464.
(22) Shibata, M. 1929 *Japanese J. Exp. Med., 7,* 247; *Shikwa Shimpo,* 22; April.

EFFECTS OF ORAL TREATMENT ON THE BLOOD PICTURE IN APLASTIC ANEMIA[1]

L. S. PETTIT, D.D.S.

Research Laboratory, Dental College, Ohio State University, Columbus, Ohio

The possible relationship between oral infection and granulopenia has been indicated by Appleton[2] and Cook.[3] Although the condition to be discussed was not diagnosed as one of granulopenia, it was a case of chronic hypoplastic anemia. In these cases all blood elements are affected, and there is granulopenia, thrombopenia and anemia. Lest the inference be drawn that we consider hypoplastic or aplastic anemia as wholly secondary to infection, we emphasize the fact that the etiology of hypoplastic or aplastic anemia is entirely unknown. The granulopenia of the present case was within the range of that noted in cases of agranulocytosis. Treatment from an oral standpoint has two objectives: (1) to attempt to prevent continuous loss of blood from gingivae, and (2) eliminate conditions in gingival crevices which might become focii of infection that would contribute to the depression of all cellular elements from the bone marrow.

On July 8, 1932, we were requested to attempt to improve the oral condition of a patient in the University Hospital because of its possible bearing on the systemic condition, and the fact that the only persistent hemorrhage occurred at the marginal gingivae. The Department of Medical Research had sought in vain for the cause of the patient's condition. The case in question, a white male patient 23 years old, gave a history of having been admitted to the University Hospital, and dismissed, several times over a period of several years for the same condition. The blood examination was made by Dr. Charles A. Doan of the Medical Research Department, to

[1] Presented at the eleventh general meeting of the International Association for Dental Research, Chicago, Mar. 18–19, 1933; *J. Den. Res.*, 1933, *13*, 209; June.

[2] Appleton, J. L. T., Jr.: Agranulocytosis, *Dental Cosmos*, 1932, *74*, 267 (Mar.) and 371 (Apr.).

[3] Cook, T. J.: Proceedings of the International Association for Dental Research; *J. Den. Res.*, 1932, *12*, 457; June.

whom we acknowledge our indebtedness for suggestions and cooperation. Smears from several gingival crevices showed an overwhelming number of Vincent organisms, especially Vincent spirochetes (*fig. 1*). This led us to attempt to reduce the flora of the gingival crevices previous to any operative procedures.

As the first step in the oral treatment, the patient was placed on a mouth-wash of sodium dichromate and boric acid,[4] and one pint of orange juice was added to his daily diet. This was continued until Aug. 1, without any other treatment; after that date it was continued in conjunction with thorough prophylaxis and local applications, to the gingival crevices, of arsphenamin in glucose. The gingival tissue, on July 30, still presented a pronounced pathological appearance (*fig. 2*). The marginal and cemental gingivae were hypertrophied and turgescent, and bled upon the least provocation. In fact there was constant seepage of blood from marginal gingivae, especially in upper anterior and bicuspid, and lower left bicuspid and molar, areas. However there had been improvement over the original condition, where practically all marginal gingivae showed seepage. A blood clot could be found in these positions at any time. The interdental papilla were ill defined, owing to tissue destruction, and the case presented a typical appearance of chronic Vincent infection. There was much sordes about the teeth; and the teeth and tongue were stained from the mouth-wash, which the patient used about three weeks previous to the taking of the photograph. The results of an x-ray examination are shown in *fig. 3*. There were no definite areas of periapical rarefaction, although the lower left first molar was badly broken down and there was apparent pulp involvement. We did not eradicate this condition because we felt the patient was not then ready for such an operation. There was some loss of alveolar border, especially in the lower left bicuspid and first molar region, and also in the upper lateral incisor areas. All losses of alveolar crest could probably be accounted for by conditions in the gingival crevices. Otherwise the alveolar process and supporting bone appeared normal. The second smear was made after the patient had used the mouth-wash and orange juice for twelve days. The bacteriological condition of gingival crevices of the upper right lateral and lower left molar are shown in *fig. 4*. There was definite decrease of Vincent organisms, with objective and subjective clinical improvement. We now felt that more severe operative procedures were permissible.

Operative treatment, begun *Aug. 1*, consisted of thorough scaling and

[4] Sodium dichromate, 5 grams; boric acid, 4 grams; aqua distilata, ad. qs., 100 cc. *Sig.:* Use once full strength, then dilute with equals parts of water and use as a mouth-wash, t.i.d.

curetting of the teeth and gingival crevices. This was done in sections, as the condition of the patient did not permit complete prophylaxis at one sitting. Details of the treatment will be omitted. It is sufficient to say that arsphenamin, in glucose, was applied to all gingival crevices three times during the operative work. On *Aug. 3*, when the patient returned, bleeding from the operated area had completely stopped; the tissues were much improved; the flabbiness of the gingivae was much reduced, and engorgement was less apparent. At this date the upper left central, lateral, and cuspid were scaled and polished. The following day, when the patient returned, bleeding had entirely stopped in the areas previously scaled. At this sitting the upper left bicuspids and molars were scaled, with very profuse hemorrhage, the subgingival deposits being unusually heavy. On *Aug. 5* the patient reported very little bleeding from the previous day's treatment, but a blood clot covered gums and teeth, causing slight hemorrhage upon removal. The upper right bicuspids and molars were scaled, with excessive bleeding. On *Aug. 9* the patient returned, reporting much bleeding following the scaling on Aug. 5. Monsel's solution had been used at the Hospital to stop the hemorrhage. The tissues were greatly improved, and the gums much tighter about the teeth. There was slight bleeding after the removal of clot from the gingival margin of the upper right bicuspid and molar regions, but this soon stopped without aid. The lower teeth were partially scaled at this sitting. On *Aug. 10* scaling and polishing of the lower teeth were completed, and all crevices treated with arsphenamin in glucose. The following day the entire mouth was much improved, and there was no bleeding from any part. Eating of coarse foods, and brushing, did not elicit bleeding, a condition from which the patient had not been free for months. Arsphenamin in glucose was again used in all gingival crevices. On *Aug. 12*, the mouth being in fine condition, the patient was dismissed from the Hospital, to return at stated intervals. Instructions to the patient for home treatment were again stressed. Smears of the upper right lateral and lower left molar crevices on Aug. 12 showed presence of some Vincent organisms, but their number had been greatly reduced (*fig. 5*). When the operative care of the mouth was started, the patient was instructed and shown how properly to brush the teeth, and to massage the gingivae with index finger and thumb. He was instructed to chew celery strings two or three times daily, to stimulate the deeper circulation. The mouth-wash and orange-juice treatment was also continued throughout the period of observation.

Fig. 6 shows variations in total number of polymorphonuclear cells (granular cells), total red blood-cells, total blood platelets, and percentage of hemoglobin. From the beginning on May 31, to July 8, there was a gradual

FIG. 1

FIG. 2

FIG. 3A

FIG. 3B

FIG. 3C

FIG. 1. Smear taken July 8, 1932, previous to use of any mouth-wash or operative procedure. Note great number of Vincent organisms.

FIG. 2. Photograph of patient's mouth to show condition before beginning of operative procedures. Note swollen condition of gingivae, and blood clots present in interdental spaces. See ulcer on lower lip.

FIG. 3. Radiographs taken when oral treatment was begun.

fall in *total polymorphonuclears* from about 2000 to slightly over 100. However in the period from July 12 to Sep. 7, there was a gradual rise to 1800. This increase in granular cells followed oral treatment, and it is reasonable to believe that it was a result of this treatment. Such an effect was indicative of the removal of toxins. We can reasonably believe that the hypo-

FIG. 4

FIG. 5

FIG. 4. Smear from gingival crevices of upper right lateral incisor and lower left molar. Note diminution in number of Vincent organisms. Smear made after patient had used mouth-wash and orange juice 12 days. No operative treatment as yet.

FIG. 5. Smear taken from same crevices as before, subsequent to prophylactic (operative) procedures, and application of arsphenamin in glucose to gingival crevices. Number of Vincent organisms greatly reduced.

thetical toxins could have been produced in the gingival crevices by the massive numbers of organisms, of which Vincent spirochete and fusiform bacillus, the predominant forms, were definitely decreased in number by the treatment. The figures for *red blood-cells* vary for the most part from 2,000,000 to 1,000,000. There was a progressive fall for about one month

previous to the initiation of oral treatment. With the oral treatment there was a gradual rise to 2,800,000, which condition was maintained until Aug. 30. It will be recalled that the first effect of the mouth-wash and orange juice was to stop marginal seepage in all but a few gingival areas; and that

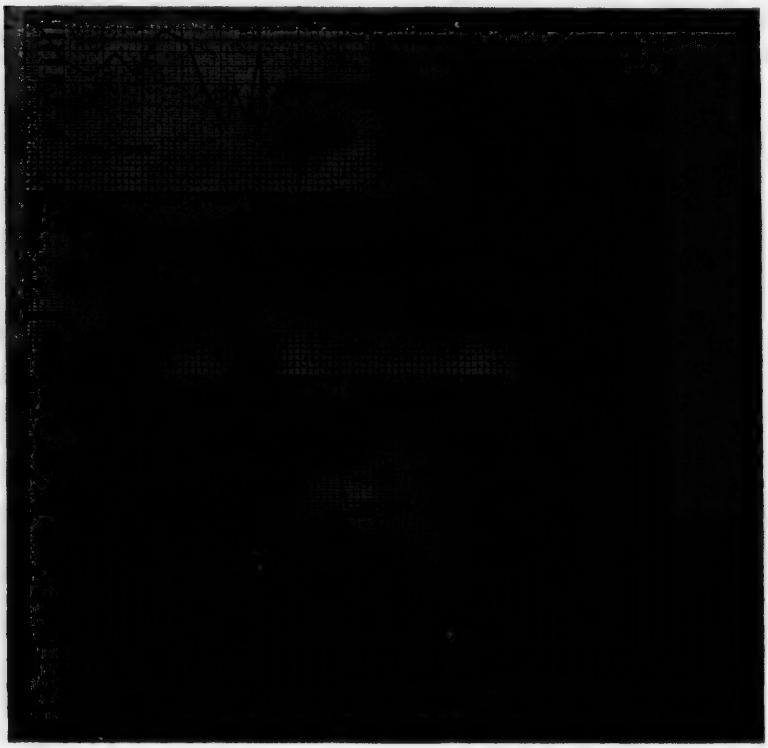

FIG. 6. Graph showing condition of blood from May 3, 1932 to Sept. 19, 1932. Total polymorphonuclear cells indicated in hundreds; total red blood-cells, in millions; platelets, in thousands; hemoglobin, in percentages.

following completion of the oral prophylaxis and arsphenamin applications, all gingival seepage stopped. The drain even though slight, coupled with lack of formative processes, had been enough to reduce the red blood-cells to about 1,000,000. Thus, the effectiveness of oral treatment is again apparent in this part of the chart. The *blood platelets* in the period previous

to oral treatment were usually less than 80,000, going down to 40,000 just before treatment was started. Subsequent to oral treatment there was a general rise in blood platelets to a new high point of about 190,000, an indication, as in the case of the granular cells, that toxins were eliminated. *Hemoglobin* started at about 30 percent; dropped to 20 percent just prior to treatment; subsequent to treatment going to 40 percent, an increase of practically 100 percent.

A single case may not warrant conclusions, but it is interesting to note how readily the tissues of the mouth responded to treatment. After the beginning of the oral work, the patient freely expressed his gratification with his improved well being, especially when all hemorrhage and irritation had been eradicated and the tissues assumed a more normal condition. It must have been a great relief, after constant bleeding and clot in the mouth, and the general filthy condition of the oral cavity. Such a mouth condition would be a hazard even to an individual in good health.

The bacteriological findings varied, some smears showing absence of Vincent organisms following use of arsphenamin in glucose. The final smears contained a few organisms, but the mouth condition clinically gave no evidence of their presence.

The patient returned to the hospital on Aug. 25 because of trouble in the hips; the mouth tissues were in good condition. The hip disorder became steadily worse, and on Sept. 19, 1932 the patient died of streptococcic cellulitis of the hips. Unfortunately we secured no photograph of the oral tissues after our treatment had been applied. Post-mortem was not permitted by relatives, and hence no tissue material could be secured.

It is our belief that the oral treatment was reflected in the blood picture, as shown in *fig. 6*, and that its effect on systemic conditions was favorable. There was no question about the local response to treatment. It was very favorable, as both the testimony of the patient and our own observations indicated.

HUMAN SALIVA

I. Critical Discussion of Former Salivary Calcium Studies and Their Value in the Establishment of Normal Standards[1]

H. BECKS, Dr. med. et med. dent., D.D.S., and W. W. WAINWRIGHT, D.D.S., M.S.

George Williams Hooper Foundation for Medical Research, and College of Dentistry, University of California, San Francisco, Calif.

I. INTRODUCTION

Throughout the recorded history of the search for the pathogenesis of paradentosis and caries, the possibility that saliva may have a damaging effect on the teeth and paradentium has been taken into consideration. However, this hypothesis, which has been discussed frequently during recent years, has not been substantiated. In any attempt to understand the way in which saliva may influence oral structures, one must have a thorough knowledge of the physiologic composition of saliva. This knowledge must be related to two problems: one dealing with paradentosis; the other, with dental caries. These two phases must be separated because caries is solely a disease of the "tooth nucleus" (Weski); in paradentosis, the entire paradentium is involved.

In the evaluation of former salivary analyses, four fundamental questions must be considered: (1) What is the physiologic composition of saliva; its physical and biochemical properties? (2) What changes occur under pathologic conditions; the effect of these conditions on physical and biochemical properties? (3) Can any deviations from usual findings be used as indications of generalized disturbance

[1] Conducted under grants made by the California State Dental Association, California Academy of Periodontology, Research Board of the University of California, and the Good Teeth Council for Children. Presented before the San Francisco Section of the International Association for Dental Research; also at the twelfth general meeting of the International Association for Dental Research, Chicago, March 17 and 18, 1934; *J. Den. Res.*, 1934, *14*, 161; June. Received for publication: April 23, 1934.

of the organism, as has been done, for instance, with blood serum, urine, etc.? (4) Is a pathologic composition of saliva *directly* responsible for, or related to, destruction of hard substances or soft tissues surrounding the teeth? The last question is of special interest, since we recognize more and more clearly that not only do certain types of paradentosis occur simultaneously with systemic diseases, but also that, according to late reports (1, 48), caries seems to be an expression of metabolic disturbance, especially of disturbed Ca:P balance. Other important questions arise concerning processes that lead to these two diseases: are they identical or similar; entirely unrelated; or opposing processes in the sense that presence of one means exclusion of the other? We refer in particular to unaccepted theories of acidosis in caries and alkalosis in paradentosis (7).

The growing interest of the medical profession in mineral metabolism and the influence thereon of the endocrine system, nutrition, and certain constitutional diseases, has led the dental profession, during the last few years, to study the inorganic composition of saliva. Results of these studies, as published by Becks (2, 3), Clark and Shell (13), Entin (15), Knoche (40), Pickerill (53), Türkheim (71, 71A) and others, usually represented averages of a comparatively small number of analyses. Very little is known about average range under physiologic conditions. Only exact biochemical studies of a large amount of material, which has not yet been gathered together, will provide adequate information about this range. In medicine, it has taken a long time to establish the physiologic range of 9–11 mg. percent for Ca in blood serum. Special difficulties attend similar work on saliva. The fact that the chemical composition of saliva is not constant throughout life must be taken into consideration. In youth, middle life, and old age, there are three different levels (2). It will be necessary to establish separately the average values, and ranges of variation, of physiologic saliva for these three levels, requiring a tremendous amount of work and uniform technic of analysis.

All the results of former analyses dealing with the different electrolytes of saliva under physiologic conditions will probably have to be revised: (a) because of recent progress in the technic of biochemical analysis, and (b) because poorly selected material has been used. Saliva was frequently taken from "clinically healthy individuals with

teeth immune to caries," or from patients with "good teeth." In selecting material, several authors gave special attention only to absence of caries. *Immunity to caries is not, however, an indication of health;* and in practically all reports there has been no indication of the presence or absence of metabolic, endocrine, or nutritional disturbances. Since it is probable that composition of saliva depends to some extent on certain changes in general metabolism, records of thorough physical examination of all cases seems to be essential, if their saliva is to be used for comparative purposes.

It is a common observation that, when authorities disagree, the fundamentals upon which their conclusions are based are usually incorrectly interpreted. In order to understand the wide variations in the recorded amounts of salivary Ca, it was necessary to review the the entire literature; to analyse and compare the different biochemical technics; and to determine whether the methods were suitable and the results satisfactory. Before we discuss the different methods employed by former investigators in salivary analysis, fundamental principles of analysis for Ca in biological material should be reviewed. It is from these fundamentals that all salivary-Ca methods have been derived.

II. FUNDAMENTAL PRINCIPLES OF Ca DETERMINATIONS

For the determination of Ca in biological fluids several methods have been introduced, nearly all of which are based on the *classical principle of precipitating Ca as very insoluble oxalate* (CaC_2O_4). This principle has been adapted for use in *gravimetric, volumetric, gasometric,* and *colorimetric* methods. The *gravimetric* method, the oldest standard procedure, is still used in some phases of biological analysis (73). However, it is not accurate when only small amounts of material are available, e.g., blood serum and saliva. In this procedure, Ca is precipitated as oxalate. This may be heated either to 300°C., where it loses one molecule of CO and is converted to carbonate; or, to white heat (ignited), when a molecule of CO_2 is also lost and it becomes the oxide. The carbonate or oxide is weighed, and Ca calculated. The first procedure is less frequently used because of danger of overheating, with consequent loss of CO_2. In recent years, the *colorimetric* method of Briggs (5), involving precipitation of Ca as phosphate, has been

used; it was modified by Roe and Kahn (61, 62) and Kuttner and Cohen (44). It has the advantage of being applicable to very small amounts, but is less accurate on portions adequate for permanganate titration (52). Another colorimetric method is that of Laidlaw and Payne (45), in which Ca is precipitated as oxalate, then as calcium alizarinate. The alizarin is freed and compared colorimetrically with a standard. This method is long, but apparently accurate. A *gasometric* method, developed by Van Slyke and Sendroy (72), depends upon measurement of the CO_2 released when oxalate is oxidized by permanganate. The margin of error, for amounts as small as those in 1 cc. of serum, is only ± 1 percent, which is considerably less than in permanganate titration. However, the maximum error of the washing technic—the same in both procedures—remains ± 3 percent. In view of this error, and the greater length of time required for the gasometric determination, permanganate titration is preferable in many instances. For the sake of completeness, two other methods no longer in use may be mentioned: *nephelometric* method, in which Ca is converted to soap and compared with a standard (47); and *iodometric* method, in which suitable reagents are added to a solution of CaO and the amount of released iodine determined by titration with a weak standardized thiosulphate solution (36A).

Introduction of *volumetric* or titrimetric principles permitted a distinct advance. Volumetric methods of two types have been developed: (1) Halverson and Bergeim (21) introduced a method in which deproteinization was substituted for the longer ashing process, and oxalate estimated by dissolving the precipitate in H_2SO_4 and titrating with potassium permanganate ($KMnO_4$). This procedure is the one most commonly used at present because of its speed and accuracy; however, it requires detailed study to achieve uniform results within a permissible range of error. (2) CaC_2O_4 may be converted to $CaCO_3$ or CaO, or a mixture of these, dissolved in standard HCl, and titrated as alkali (19, 22, 70). These methods have been offered as improvements over the permanganate titration, but the procedure has been lengthened and complicated by extra steps without reduction of experimental error. The first of these two volumetric methods is one of the most fundamental procedures for Ca determination. Most of the methods now in use, which are modifications of it, include

improvements principally in the deproteinization and washing procedures, which were the main sources of error. So far as deproteinization is concerned, Kramer and Tisdall (42) substituted the more convenient trichloracetic acid precipitant of Greenwald (20) for the picric acid used by Halverson and Bergeim. The possibility of precipitating Ca in the presence of protein has been discussed by a large number of authors, who concluded that recovery of Ca is satisfactory (10, 11, 14, 22, 41, 54, 70). However, Van Slyke and Sendroy showed that presence of proteins measurably inhibits precipitation of calcium oxalate, causing *low recovery*. On the other hand, any organic matter present during titration neutralizes permanganate, *causing high values*. Furthermore, it is believed by Tingey (69) that protein adsorbs oxalate from wash liquid containing CaC_2O_4, leading also to *high values*. For these reasons, it has been generally accepted that complete precipitation and satisfactory recovery of Ca can be assured only by preliminary removal of protein.

In the analytical procedure, the *washing technic* presents the largest number of obstacles. Three phases are involved: (a) technic of washing (centrifugation, filtration); (b) selection of wash liquid; (c) number of washings and volume of wash liquid used. Refinement of these procedures has permitted determination of smaller amounts of Ca. Halverson and Bergeim (21) employed the centrifuge to separate the precipitate, and called for one washing with approximately 50 cc. of cold distilled H_2O. They suggested use of a siphon with an upturned tip for removal of supernatant fluid, which by this means may be drawn off with minimum disturbance of the precipitate, thus avoiding loss of crystals. Clark (11) used similar technic. These procedures required amounts of serum up to 5 cc., and the experimental error ranged as high as ±5 percent. Kramer and Tisdall (41) found that the error from the washing technic could be decreased by using less wash liquid, and dilute ammonia instead of distilled H_2O, because CaC_2O_4 is less soluble in ammonia. However, they washed three times with 4-cc. amounts of 1:50 NH_4OH, and removed the supernatant fluid by siphoning with an upturned capillary device. This modification had the advantage of needing only about 2 cc. of serum for a single determination, but the error still varied between ±3–5 percent. Tisdall (69A) substituted decantation for siphoning, to re-

move supernatant fluid more completely, and decreased the washings to *two* with 4-cc. amounts of dilute ammonia. Clark and Collip (10) used more thorough decantation, and thereby reduced the washings to *one*, with 3-cc. of 1:50 NH_4OH, the small volume being permissible because of more complete removal of mother liquid. The small amount of $(NH_4)_2C_2O_4$ remaining compensates for the slight loss of precipitated CaC_2O_4 by solution in the wash liquid, or by decantation. This method requires 2 cc. of serum also, and occasionally errors greater than ± 5 percent occur. Van Slyke and Sendroy (72) found the most reliable technic to be *two* washings with 3 cc. each of 1:50 NH_4OH in a conical 15-cc. centrifuge tube, the supernatant fluid being drawn off with an upturned capillary pipette (leaving about 0.3 cc. of liquid) to avoid disturbing the precipitate. This method permits analysis of 2-cc. amounts of serum with an experimental error not greater than ± 3 percent. Stanford and Wheatley (68) suggested saturating the wash liquid with CaC_2O_4 to prevent loss of precipitated Ca, by solution, when large amounts of wash liquid are used. This procedure, found very satisfactory, is recommended by many investigators. The principal source of error in these methods—all depending on use of a centrifuge for washing—is the possibility of loss of precipitate on decanting or siphoning, because, after centrifuging, crystals of CaC_2O_4 tend to float on the surface and to creep up the sides of the tube and siphon. Recognizing the difficulties of the centrifuge method, Kirk and Schmidt (38, 39) and Fiske and Adams (19) adapted the use of filters by devising *micro*-filters suitable for amounts of Ca equal to those in 1 or 2 cc. of serum. The filter used by Kirk and Schmidt replaces the centrifuge technic, thus eliminating the possibilities of loss mentioned above. The procedure is so designed that permanganate titration may be employed. Their use of 1:50 NH_4OH saturated with CaC_2O_4 as wash liquid permits thorough washing without loss of Ca by solution into the wash liquid. The maximum error can be kept within the range of ± 3 percent.

Selection of one of these procedures for analysis of biological material (serum or saliva) depends largely on the amount of material available for analysis and the degree of accuracy required. In any research, methods having the smallest range of unavoidable error must be given preference. For this reason, the titrimetric methods of Van

Slyke and Sendroy and of Kirk and Schmidt seem to be the most advanced and suitable. However, any selected procedure will include some arbitrary features, which must be followed closely during an entire series of examinations, if the results are to be useful for comparative purposes.

III. TECHNICAL APPROACH OF FORMER INVESTIGATORS IN THE ANALYSIS OF SALIVARY Ca, AND CRITICAL DISCUSSION OF THEIR WORK

Review of the literature of salivary-Ca analysis reveals many different procedures. In numerous instances, the work has been surprisingly inadequate. Authors who followed related procedures will be considered together rather than in chronological order. On this basis, they fall into three groups, as shown in table 1.[2] The earliest investigators to whom we were able to find reference were Frerichs (19A), Hammerbacher (23), Hammarsten (24), and Jacobi (34). They reported their values as percent of Ca in 100 grams of ash, calculated from the weight of CaO obtained. Application of these *macro*-methods was limited, because of the large amount of material required, sometimes even necessitating use of pooled samples. Because of the nature of the analytical procedure, limited number of analyses, and units in which the results were expressed, comparison with recent work is hardly possible. Accordingly, the work of these authors has been omitted from *table 1*, but their names are mentioned here because the credit of being pioneers in the study and analysis of human saliva goes to them.

Group I. A. One of the earliest investigators of salivary Ca was Röse (63), who tried to solve, with primitive methods, problems concerning the influence of nutritional habits and composition of drinking water on saliva in its relation to dental decay. He reported many analyses made by a gravimetric method, and has been classified with the authors in Group IA because their methods are similar. His values fall within a range of 5.5 to 22.4 mg. percent. However, no information was given as to amount of saliva used; rate of flow, age, and health of patient; whether resting or activated saliva was selected;

[2] See also *tables 3* and *fig. 1* for additional related data.

TABLE 1

General characteristics of methods used in former salivary-Ca analyses

1	2	3	4	5	6	7	8			9	10	11
GROUP	AUTHOR	YEAR	SALIVA FOR SINGLE DETERMINATION	PRELIMINARY CENTRIFUGATION?	PRELIMINARY METHOD OF REMOVING ORGANIC MATTER	INFLUENCE OF PRELIMINARY TREATMENTS ON RESULTS	WASHING TECHNIC: Liquid and amount	WASHING TECHNIC: Removal of wash liquid by:	WASHING TECHNIC: Washings:	CALCIUM ESTIMATED BY:	METHOD CHECKED ON TEST SOLUTION	ANALYTICAL PROCEDURE (SUMMARY)
			cc.						no.			
I A	Rõse	1905	?	?	Ashed in platinum	?	?	?	?	Weighing	?	Unsuitable
I A	Bunting and Rickert	1915	25	?			Dist. H_2O ? cc.	Paper filtration	?		?	
I A	Bunting and Wixon	1917	25	?							?	
I A	Rickert and Palmerlee	1924	25	?							?	
I	Ruthrauff	1923	25	?							?	
I	Broderick	1920	?	?	?		?	?	?	*Counting* CaC_2O_4 crystals in hemocytometer—recorded as Ca index	?	
I B	Janssen	1926	12.5–25	No	Deproteinized	High	Dist. H_2O ? cc.	Paper filtration		0.1 *N* permanganate titration	?	
I B	Hanssen	1933	2.5	No		High	1:50 NH_4OH: 5 cc.	Glass filtration (Hanssen)	3	0.01 *N* permanganate titration	Yes	
II A	Spencer-Payne	1924	?	?	?	?	$(NH_4)_2C_2O_4$ 0.1%: 3 cc. (Laidlaw-Payne)	Siphoned	1	Alizarin freed, and compared colorimetrically with standard	?	Suitable (?); incompletely reported
II A	Pattison	1926	?	?	?						?	
II	Knoche	1927	?	?	?		?	?	?	?	?	
II	Pattison	1926	?	?	?		1:50 NH_4OH: 4 cc. (Tisdall)	Decanted	2		?	
II B	Entin and Eiger	1927	?	?	?	?	1:50 NH_4OH: 4 cc. (Kramer-Tisdall)	Siphoned	3	0.01 *N* permanganate titration	?	
II B	Entin	1929	?	?	?						?	
II B	Hawkins	1928	?	?	?						?	
II B	Price	1932	?	?	?						?	
II B	Fetterly and Maughan	1931	?	?	Deproteinized		1:50 NH_4OH = 3 cc. (Clark-Collip)	Decanted	1		?	
III A	Clark and Shell	1927	4	No	Ashed in platinum	High	Dist. H_2O 35 cc. (Clark)	Siphoned	1		Yes	Suitable; completely reported
III A	Clark and Levine	1927	4	No							Yes	
III A	Roskin	1928	4	?							?	
III A	Leonard	1928	10	No							Yes	
III A	Hubbell and Bunting	1932	4	?			1:50 NH_4OH: 3 cc. (Clark-Collip)	Decanted	1		?	
III	Ferris	1923	3.2	No	Oxidized	Low	1:50 NH_4OH: 4 cc. (Kramer-Tisdall)	Siphoned	3	0.01 *N* permanganate titration	?	
III	Becks	1929	2	Yes							?	
III B	Horton, Marrack, and Price	1929	?	?	Deproteinized	High	H_2O: sat. CaC_2O_4: 35 cc. (G. W. Clark)	Siphoned	1		?	
III B	Karshan, Krasnow, and Krejci	1931	5	No			Acetone: 0.5 cc. + 1:50 NH_4OH: 3 cc. (Karshan-Krasnow-Krejci)	Decanted	2		Yes	
III B	Krasnow	1932	5	No							Yes	

or check of the method on a test solution. Since the procedure employed is inadequate, when compared with present methods, the results cannot be considered dependable.

In 1915, Bunting and Rickert (8) reported analyses of saliva by a gravimetric method: 25 cc. of saliva were ashed in platinum; ash dissolved in acetic acid; Ca precipitated as oxalate; precipitate washed on filter with distilled H_2O, dried, ashed in platinum to CaO; and ash *weighed*. This method is subject to comparatively large errors because of smallness of the amounts of CaO weighed (1.5 – 7 mg.), and loss of CaC_2O_4 in washing with H_2O. It is possible also that a large error was introduced by conversion of CaC_2O_4 to $CaCO_3$ and then incompletely to CaO. If any $CaCO_3$ remained, it increased the weight, giving high results; even a small *residual* amount would be significant. Leonard (46) was unable to get comparable results on known amounts of Ca by this method, and reported an experimental error of as much as 25 percent, far beyond the permissible limit. Furthermore, 25 cc. of saliva were required, which does not favor duplicate analysis, nor analysis of saliva of patients who have very slow rates of flow. Subsequently, Bunting and Wixon (9) and Rickert and Palmerlee (60) reported results by this method, which, for activated saliva from patients who showed "distinct caries immunity," varied between 3.5 and 12.0 mg. percent of Ca. Bunting and Wixon, in a second paper, noted that the results were somewhat lower throughout than in the first report, and referred to the possibility that this was due to change in technical assistance. Ruthrauff (65) reported the examination of activated and resting salivas of persons immune to caries (6 cases). The results fell between 9.15 and 17.1 mg. percent of Ca in resting saliva. The method of analysis was not given. However, the results were reported as CaO per 25 cc., and were apparently obtained by the method employed by Bunting and Rickert, and hence are subject to the same criticism. He did not report the health of the subjects. None of these authors gave data on rate of flow nor on checks on test solution.

B. The authors of this sub-group used dissimilar methods that were also inadequate. Broderick (6) is the only one to report salivary studies by the Blair-Bell method (4), involving precipitation of Ca as oxalate, the crystals of which are counted in a hemocytometer and the

result given as Ca-index. This index cannot be converted into terms of mg. per 100 cc. The method does not control the size of the crystals, a variable that vitiates the results.

Janssen (35, 36) determined Ca in saliva from over 100 persons. He failed to state (a) whether resting or activated saliva was used; (b) age and health of patients; (c) whether his method was checked with a test solution. His procedure, as described in detail: 12.5 to 25 cc. of saliva were washed into a flask; proteins precipitated by addition of HCl, NH_4OH, and acetic acid. Mixture was heated; Ca precipitated as oxalate; after 24 hours, precipitate washed on paper filter with distilled H_2O, dissolved in hot H_2SO_4, and oxalate titrated with 0.1 *N* permanganate ($KMnO_4$). All of Janssen's results were calculated as mg. of Ca *per hour*; average for men, 17.2; for women, 19.4. In one case where sufficient data were given, Ca, recalculated by us, was 26.4 mg. percent. Although Janssen used a volumetric procedure, which was an improvement over gravimetric methods, it is subject, because of the washing technic, to larger errors than the centrifuge methods. By washing the precipitate on a filter with distilled H_2O, a comparatively large amount of CaC_2O_4 was lost by solution. However, Janssen substituted deproteinization for dry ashing, and the oxalate was titrated with 0.1 *N* $KMnO_4$ instead of being weighed as CaO (Group IA).

Hanssen (25) suggested a new method, introducing glass filters for the washings. He precipitated with an acetic-HCl mixture. From portions of filtrate corresponding to 2-cc. samples of saliva, Ca was precipitated as oxalate without adjustment of pH (66). A sintered glass-filter was used to separate the precipitate, which, washed with three 5-cc. portions of plain 1:50 NH_4OH, was dissolved in hot 1 *N* H_2SO_4, and oxalate titrated with 0.01 *N* $KMnO_4$. The use of a filter instead of a centrifuge in the washing technic offers several advantages. However, it is doubtful whether the size recommended by Hanssen should be used in the analysis of saliva. It is quite possible that as the sintered glass-filter in the Alihn tube 15aG4 has a diameter of 2 cm., a significant amount of wash liquid is held in the pores of the filter. The size of the tube (10 cm. high by 2 cm. in diameter) and filter disk are such as to make thorough washing difficult. Other errors are introduced in the procedure. When 5 cc. of saliva are added to 5 cc. of

acetic-HCl mixture, the resultant volume falls short of 10 cc. This is a common phenomenon with acids in high concentrations. Although when this mixture is boiled there is resultant loss of H_2O, Hanssen fails to mention the change in volume or the means for its correction. These two factors induce high findings. The 15-cc. volume of plain

TABLE 2

Analyses of test solution containing 10.0 mg. percent of Ca; by (A) Hanssen's method and (B) Kirk and Schmidt's method; showing loss of Ca when plain wash-liquid is used

	DATE	AMOUNT OF SOLUTION	CALCIUM PER 100 CC.	
			Found	Deviation
		cc.	*mg.*	*per cent*
A.	9/5	2	11.25	+12.5
			10.6	+6.0
			10.97	+9.7
			11.15	+11.5
		2	10.78	+7.8
			10.68	+6.8
			11.15	+11.5
			10.48	+4.8
	9/8	2	10.71	+7.1
			10.81	+8.1
	9/8	2	10.15[1]	+1.5
			10.42[1]	+4.2
B.	9/4	2	9.9	−1.0
			9.46	−5.4
			9.66	−3.4
			9.56	−4.4
	9/5	2	9.71	−2.9
			10.1	+1.0
			9.71	−2.9

[1] Volume adjusted after boiling, and before filtering out protein precipitate.

wash-liquid recommended by Hanssen dissolves some Ca, causing significant loss. This has been shown by the work of Van Slyke and Sendroy (72), and others. It is illustrated by the results shown in section B of *table 2*. These were obtained in tests of the Kirk-Schmidt method in which plain wash-liquid was substituted for that saturated with CaC_2O_4. With one exception, the results were low, varying from −5.4 to +1.0 percent from the theoretical value. At the end of the

washing, Hanssen's procedure calls for a clean tube in which to collect the acid solution that dissolves the precipitate on the filter disk. This may cause low results, as there is no way to determine at the time whether crystals of CaC_2O_4 remain in the already well-washed tube in which the original precipitation was made. Hanssen reported analyses of a test solution of calcium acetate containing 9.76 mg. percent of Ca, the results ranging between 9.27 and 9.59 mg. percent. The recovery apparently was always low, the maximum variation from the theoretical value being −5.0 percent; the minimum, −1.7. Eighteen tests on one sample of saliva gave him values between 7.6 and 8.4 mg. percent of Ca, the average being 7.94, the percentage difference from the mean varying from +5.8 to −4.3, which is so great as to suggest the presence of an unusual experimental error.

In section A of *table 2*, the results of tests in our laboratory, by Hanssen's method on known solutions, are from +4.8 to +12.5 percent higher than the amounts of Ca present. Hanssen's analyses of test solutions were, in every case, lower than his theoretical value (from −1.7 percent to −5.0), whereas we obtained only high values. This suggests that among the sources of error noted above, those related to (a) uncontrolled volume-factors, causing increase in concentration, and (b) difficulties in washing, because of size of apparatus, have greater influences on results when the technic is carried out as published. When the volume, after boiling and just before filtering precipitated proteins, was adjusted to 10 cc., the two tests gave values +1.5 and +4.2 percent higher than the amount of Ca present, a reduction of error indicating the influence of this factor alone. With these errors in mind, it is impossible to conclude that this method is accurate. Hanssen's procedure does not present an improvement over the standard centrifuge technics, and cannot be accepted for use in salivary analysis. However, the principle, as such, is excellent, as Kirk and Schmidt have shown. Hanssen gave no information as to the type of saliva examined (activated or resting), rate of flow, age and health of subjects.

All methods in Group I are subject to large errors. The results obtained with them cannot be accepted as accurate or sufficiently dependable for comparison with subsequent work.

Group II. This section deals with the work of authors who may

have used suitable methods and correct analytical procedure, but who failed to give a detailed description of them (*table 1*). Most of the authors in this group referred to technics developed for analysis of blood serum, and applied them to saliva without indicating the preliminary treatment, which, as will be explained, must be different from that of serum. Spencer-Payne (67) reported determinations of salivary Ca by the Laidlaw-Payne (45) colorimetric method. Ca was precipitated as oxalate, washed once in a centrifuge with 0.1 percent $(NH_4)_2C_2O_4$, and dissolved in 0.5 cc. of *N* HCl. The solution was diluted to about 10 cc. and excess of alcoholic solution of alizarin added (1 cc. of 1:1000 solution when 0.1 mg. of calcium is present), to precipitate Ca as alizarinate. After standing, this was filtered in a Gooch crucible on asbestos with suction; washed with dilute ammonia; alizarin freed with 1 cc. of strong oxalic acid in 50 percent alcohol; crucible washed with 95 percent alcohol; and solution of alizarin, diluted to 50 cc., compared in a colorimeter with a standard. Spencer-Payne did not indicate the values obtained. He said: "Indeed the calcium content seemed to vary not only with the individual but also from day to day; and indeed at different periods of the day. Furthermore, there was certainly no clear relation between the amount of calcium and the liability to caries." He gave no information concerning kind and rate of flow of saliva, age and health of patients, and tests on known solutions. This colorimetric method has also been used by Pattison (51), who reported that results compared favorably with duplicates by Tisdall's method. Pattison omits much essential information concerning the preliminary treatment and the methods used. He does not state what a normal value would be, but reports simply that, by diet high in "calcifying vitamin," salivary Ca was raised from a low value (average 3.07 mg. percent) to a higher one (6 and 12). These cases were all hospital patients (children) with the exception of twenty persons (adults and children) whose histories were not given, although source of drinking water was noted. Their values, reported as averages for four groups, were respectively 8.07, 10.61, 7.19, and 9.34 mg. percent of Ca. He stated there was no relation between decay and rate of flow; did not report checking the method on a test solution. The Laidlaw-Payne (45) colorimetric method contains steps similar to the usual centrifuge-washing technic and, in addition,

precipitation, washing, and solution of the alizarinate. Even if the comparison with the standard is very accurate, the errors before that final step are about the same as in the usual volumetric method. Thus, this procedure offers no improvement on the accuracy or convenience of the shorter volumetric methods. Since the work of Spencer-Payne and Pattison (1924–26) there has been no reported use of the Laidlaw-Payne method for salivary analysis.

From 1927 to 1932, Hawkins (26, 27, 28, 29, 30), Entin and Eiger (15), Entin (15A), Knoche (40), and Price (55, 56, 57, 58, 59) published results of salivary analysis, but did not give detailed descriptions of their methods. Hawkins, Entin and Eiger, and Price, stated merely that they used the Kramer-Tisdall technic. Hawkins (27) used Matthews' modification of this method, but no reference was given for Matthews, and there is no record of the adapted method as used by Hawkins. The recorded results vary so much from those of most other authors as to suggest unusual error, even though "thousands" of analyses were made. The range of salivary Ca for those "immune to caries" varied from 15.0 to 145.0 mg. percent.[3] He gave no information as to rate of flow, age and health of patient, and checks on test solution. He did not even indicate just what technic was used for the collection of the saliva samples. He said that "it is important to obtain *active* saliva, not resting saliva." Also: "It is important that at least one hour elapse after eating before a specimen is obtained, and that no exercise be taken for a considerable length of time, or the results will be erroneous" (27, p. 789). Presumably, he used activated saliva, but the method of activation was not indicated. In Knoche's article presenting analyses of 76 cases, we do not find any reference to his analytical procedure. His values ranged from 12.2 to 17.8 mg. percent of Ca. Entin and Eiger, reporting studies on 138 patients comparatively immune to caries (using resting saliva), found an average of 15.9 mg. percent of Ca. In a later paper, Entin reported analyses for 121 patients immune to caries, with a range of 5 to 33 mg. percent—average, 16.2. They omitted rate of flow, age and health of

[3] In a personal communication, Hawkins has indicated that he found 0.15 to 1.45 mg. per 1 cc., which is 15.0 to 145.0 mg. percent. It is obvious that this range was found by Hawkins through miscalculation, and must therefore be disregarded in further discussions.

patient, and checks on test solution. Even though it be assumed that all these authors prepared their material with the utmost care, it cannot be determined whether modifications of technic made their results low or high. This is important, if their values are to be compared with those obtained by the authors in Group III. Price, who has done considerable work on Ca metabolism in relation to dental diseases, reported Ca analyses for saliva in 25 cases. The values ranged between 4.5 and 10.0 mg. percent, averaging 6.0, and were accompanied only by this brief comment: "It happened that no extreme values appeared in this group." It was not stated whether the saliva was activated or resting, and no information was given regarding preliminary treatment, rate of flow, age and health, and checks on test solution. The method used was "adapted" to saliva (57) from that of Kramer and Tisdall (41). In other publications (58, 59), many more analyses of salivary Ca were mentioned, but the original values were not given, the results being recorded in terms of percent of Ca taken up or lost when saliva was shaken with bone chips. In another paper, changes in the diffusible Ca of saliva upon irradiation were reported (55). It is impossible to calculate the original values in mg. percent of Ca from any of these results. Furthermore, Price mentioned in one article (57) that formalin was added to preserve the sample when it had to be shipped a distance. This formalin, and the delay before analyzing, may or may not have influenced the results obtained. Fetterly and Maughan (18) reported results for salivary Ca in 52 cases. They deproteinized with acetic acid, and followed Clark-Collip's method. No information was given regarding amounts of saliva used for single determinations; rate of flow; whether the saliva was activated or resting; and apparently no tests of the method on known amounts of Ca were made. The recorded results of 26 cases with "excellent teeth" ranged from 3.2 to 4.6 mg. percent of Ca.

In summarizing the work of the authors in Group II, it is found that they employed methods which have been accepted in medicine for analysis of serum and other biological material. However, they omitted to mention the preliminary treatment of saliva and essential parts of the methods. In addition, in four instances (Spencer-Payne, Hawkins, Price, and Fetterly and Maughan) the authors did not men-

tion whether they used resting or activated saliva.[4] *Table 3*, indicating technical approach, material, and results obtained by the authors in Groups II and III, enables the reader to see at a glance the outstanding weak points in their work. Column 7 indicates that physical examinations were not made for the patients in Group II. The authors recorded only that the teeth were "immune" or "comparatively immune" to caries, or that the patient had "good teeth." Pattison failed to record the health of one group of 20 patients—the only ones among the total of 71 cases not reported as pathologic. In the entire group, he reported only two factors related to health: nutrition and tuberculosis. With the exception of Pattison, no author in Group II paid any attention to rate of flow (column 5). Age of patients (column 6) was reported only by Pattison, and Fetterly and Maughan. A very important factor in judging reliability of methods and results is the attitude of investigators regarding tests of the methods they used. Column 10 of *table 1* shows that none of the authors in Group II checked the methods on test solutions.

In comparing the results for salivary Ca, we find that eight investigators in Group II (*table 3*) analyzed saliva of more than 561 patients. These do not include the analyses of Spencer-Payne, who did not report their number. His work has been widely discussed, but all essential data are missing. The results on these 561 patients range between 3.2 and 33 mg. percent of Ca, which variation is far too wide for any comparative studies. Price, and Fetterly and Maughan, obtained results for their 66 patients that were all *below* 10 mg. percent; Knoche reported about 76 cases, all ranging *above* 12. The rest of the authors in Group II reported very wide variations, with results both very low and very high. Average values were not stated by a number of authors in Group II. Entin and Eiger gave only the average of their results without indicating the range, but in another publication, Entin gave the range and average covering apparently the same material. These wide variations were apparently due to the fact that the methods employed were not tested, thus leaving open the probability of technical errors; or that the material was not taken from individuals free from metabolic disturbances.

[4] It has been found necessary to limit the analysis to "resting" saliva, to be sure that no influence can be exerted by any exogenous stimuli. In a later communication, factors that influence composition of saliva will be reported in greater detail.

TABLE 3

Material and results of former salivary-Ca analyses[1]

1	2	3	4	5	6	7	8	9
GROUP	AUTHORS	NO. OF CASES	RESTING OR ACTIVATED SALIVA	RATE OF FLOW	AGE OF PATIENTS	HEALTH OF PATIENTS	RESULTS	
							Range mg. %	Average mg. %
I	*See table 1*	Data omitted here because unsuitable methods were employed.						
II	Spencer-Payne	?	?	?	?	?	?	?
	Pattison	20	Resting	Yes	Adults Children	?	8.07–10.61[2] 7.19– 9.34	
	Knoche	76	Resting	?	?	Caries immune	12.2–17.8	?
	Entin-Eiger	138	Resting	?	?	Clinically healthy individuals	?	15.9
	Entin	121	Resting	?	?	Immune to caries	5 –33	16.2
	Hawkins	140	?	?	?	Immune to caries	15.0–145.0	?
	Price	25	?	?	?	?	4.5–10.0	6.0
	Fetterly-Maughan	41	?	?	"Normal" college women	"Good teeth"	3.2–4.6[3]	
III	Clark-Shell	5	Resting	Yes	22, 31, 36, 42, 49	Good[4]	4.6–11.0	?
	Clark-Levine	3	Resting	?	?	?	4.0– 5.6	?
	Roskin	17	Resting	?	Children	Diabetics	3.7– 7.75	?
	Leonard	1	Resting	?	39	Immune to caries	6.4– 6.56	—
	Hubbell-Bunting	22	Activated	Yes	7–16	No active caries	2.2– 7.5	?
	Ferris	1	Resting	Yes	29	Paradentosis	8.4– 8.9	—
	Becks	24	Resting	Yes	8–71	Good[4]	7.5–17.5	12.1
	Horton-Marrack-Price	86	Activated	?	3–12	Caries free	6.0–14.0	?
	Karshan-Krasnow-Krejci	17	Activated	?	15–25	Caries immune	4.9– 9.2	?
	Krasnow	11	Resting	?	19–21	Caries immune	?	4.9

[1] Results from material used for comparison with pathologic cases. Heavy lines indicate deficient data.

[2] Averages of four groups.

[3] Extreme averages of five groups.

[4] No metabolic disturbances, and immune to caries.

In again summarizing the work of the authors in Group II, it can be stated further that their results may be dependable and useful for the establishment of normal standards, but this is uncertain because of the incompleteness of their reports. Thus, Entin, using the Kramer-Tisdall method, reported results ranging from 5 to 33 mg. percent of Ca—a range never found by another author. Possibly he omitted centrifugation and deproteinization—omissions that favor very high values, as will be demonstrated (*table 7*, group 4). This doubt arises particularly when we note that Kramer and Tisdall suggested two methods, both in 1921: in the first (41), they did not deproteinize; in the second (42), deproteinization was used. Which did Entin employ?

Group III. The authors in this group gave thorough descriptions of every step in the analytical procedures. However, a number of deficiencies make the reliability of their results rather doubtful.

The procedure followed by Clark and Shell (13) and Clark and Levine (12) called for ashing in platinum at 600–650°C. The ash solution in HCl was filtered, and aliquots corresponding to 4 or 5 cc. of saliva were taken. Ca was precipitated as oxalate in a conical 50-cc. centrifuge tube at pH = 5.6 to 5.8. After centrifuging, the precipitate was washed once with 25 cc. of cold H_2O. A siphon was used to remove supernatant fluid. Permanganate titration was carried out according to Clark's original method (11). Clark and Levine omitted information on rate of flow, and age of subjects. Their results ranged from 4.0 to 5.6 mg. percent of Ca. Clark's volumetric method for Ca is somewhat long, owing to the dry ashing, and subject to the experimental errors of centrifuge washing, particularly since he used a large volume of distilled H_2O instead of dilute NH_4OH as wash liquid. The results reported by Clark and Shell on the saliva of five "physically sound men under different conditions" fall between 4.6 and 11.0 mg. percent of Ca. Roskin (64), following the procedure of Clark and Levine, on saliva in 17 cases of diabetic children receiving a special diet and having no active caries, ranged from 3.7 to 5.7 mg. percent of Ca. He did not mention rate of flow. Leonard (46) determined Ca of saliva in one case, by his modification of the Clark-Collip method for blood serum. He stated that the method for blood was not suitable for saliva because of the content of mucin, which cannot be fil-

tered or centrifuged off, nor completely destroyed except by ashing. 10 cc. of paraffin-activated saliva were ashed in platinum. The ash was dissolved in HCl, and Ca precipitated as oxalate after adjusting pH with bromphenol blue. The precipitate, washed once with 3 cc. of 0.56 percent NH_4OH, was dissolved in 1 *N* H_2SO_4 and titrated with 0.01 *N* $KMnO_4$. Leonard's tests with known amounts of Ca corresponding to that in 10 cc. of saliva gave results that came within ±2 percent of the known amount. The method apparently gives excellent results, but is long and requires a large sample. Leonard's paper presents only the reports of tests on his method and a brief review of the literature up to that time on salivary Ca. His results in the one case averaged 6.5 mg. percent of Ca. He did not present any data on rate of flow. Hubbell and Bunting (32) reported salivary-Ca studies on 92 cases. Activated saliva was ashed in platinum, and portions corresponding to 4 cc. of saliva were used for Ca determinations by the method of Clark and Collip. The results for 22 cases "with no active caries" varied between 2.2 and 7.5 mg. percent of Ca. Ferris (16, 17) also eliminated organic matter by ashing in platinum. He used the dissolved ash of 3.2 cc. according to the technic of Kramer and Tisdall (42) without preliminary centrifugation. Ferris gave 10 mg. percent as the "usual average" for salivary Ca. However, he reported actual Ca results for one case only, the value being 8.4 mg. percent on one occasion and 8.9 a month later. Horton, Marrack, and Price (31) used the Clark-Shell method (13), with Stanford and Wheatley's (68) addition of calcium oxalate in the wash liquid (apparently H_2O). They determined salivary Ca in 835 cases. The results for those having no caries ranged from 6 to 14 mg. percent. Data on rate of flow were omitted.

In 1929, Becks (2, 3) examined 25 subjects who were in good health and had been caries-immune for at least a year previous thereto. After preliminary centrifuging of the sample and precipitation of Ca as oxalate, he oxidized the remaining organic matter with fuming HNO_3 and precipitated Ca a second time with ammonium oxalate, thus eliminating interference by remaining organic matter in titration with permanganate. Even though Becks might have lost a certain percentage of Ca through preliminary centrifugation, his values, ranging from 7.5 to 17.5 mg. percent, and averaging 12.1, are higher than

those of some of the other authors. He did not report checks on test solution. Karshan, Krasnow, and Krejci (37) reported results of salivary analysis in 44 cases. They used a modified Tisdall procedure (43A). Proteins were precipitated with trichloracetic acid and the mixture centrifuged. Ca was precipitated as oxalate from a volume of supernatant fluid, at pH 5.9, corresponding to 4 cc. of saliva. Washings were made twice with 3-cc. portions of 1:50 NH_4OH plus 0.5 cc. of acetone. The supernatant fluid was decanted. Acetone in the wash liquid so changes the surface tension that the tendency for crystals of CaC_2O_4 to float during centrifugation is reduced. The results for young adults "immune to caries" ranged from 4.4 to 9.2 mg. percent. They omitted all mention of rate of flow. Krasnow reported further studies by this method on 25 cases (43). For 11 "caries-free" cases, values averaged 4.9 mg. percent of Ca. She also omitted to indicate rate of flow.

The work reported by the authors in Group III (*tables 1* and *3*) is more dependable than that of authors in Group II, because the methods and data have been given in complete form. However, the results of several cannot be accepted for the establishment of standard values for the following reasons: (1) Hubbell and Bunting, Horton, Marrack, and Price, and Karshan, Krasnow and Krejci, used activated instead of resting saliva. (2) Very little attention was paid to rate of flow in its relation to concentration of Ca. Becks, and Hubbell and Bunting, were the only ones who indicated that there seems to be a definite relationship. However, in the case of Hubbell and Bunting statistical data are missing. It is extremely important to know the rate of flow of saliva in order to interpret the Ca findings.[5] Clark and Shell, and Ferris, also recorded the rate of flow, but did not interpret it. (3) The seventeen analyses by Roskin were made on diabetic patients. Ferris' only analyses were made on a paradentosis patient. We do not find any reference to the health of the three patients examined by Clark and Levine. (4) With the exception of Clark and Shell, and Becks, none of the authors in Group III had their patients examined from a metabolic standpoint. (5) Only Clark and Shell, Clark and Levine, Leonard, Karshan, Krasnow, and Krejci, and Krasnow, of the

[5] Statistical data will be presented in another communication.

authors in Group III, reported checking their methods on test solutions. (6) Krasnow gave only an average of her results for eleven patients without indicating the range. Most of the other authors in Group III gave only the range and not the average. In some instances, the averages could not be calculated because necessary data were missing. In critically surveying Group III, it is found that, with the exception of Clark and Shell, every author failed in at least one respect. The deficiencies are indicated by heavy lines about the various items in *table 3*.

The authors in Group III analyzed the saliva of 187 patients for comparative studies and their results varied from 2.2 to 17.5 mg. percent of Ca. It is a striking fact that all of these results, excepting those of Becks, and Horton, Marrack, and Price, fall below 10 (77 patients). The range of variation is very much smaller than that for Group II. It seems probable that Group III came closer than Group II to the actual Ca level. However, from an objective standpoint, the results obtained from pathologic material (Roskin, Ferris) cannot be considered for our purpose. Since we do not know how far failures by the other authors of Group III (*table 3*) affected their results, it is better not to use their data for the establishment of standards. Only the results obtained by Clark and Shell can be accepted as satisfactory material for normal standards. Of course, it is true that the results obtained by each author in Group III *may* fall within a normal range in spite of any errors or omissions. From an analytical standpoint even the Clark-and-Shell results may be high because of the preliminary treatment of saliva (*table 7*). This may be true for other authors in Group III (excepting Becks), because they removed organic matter either by ashing or deproteinization without preliminary centrifugation.

If we compare the procedures used by all authors in Group III (excepting Becks), with those of the originators of the various Ca methods, we find that the former *failed* to remove cells and foreign material by preliminary centrifugation. In analysis of blood, cells are removed by this method. In other words, all altered the original methods without considering the probability that the modifications considerably influenced the results. It is possible, therefore, that their results deviate from true values because of the influence of par-

ticular preliminary treatments. The results reported by all who omitted preliminary centrifugation but applied deproteinization (including those using dry ashing) were possibly too high because of inclusion of débris.

In *fig. 1* variations in results for resting and activated saliva have been recorded graphically. A third group has been added, to include results for which type of saliva was not indicated. This graph shows

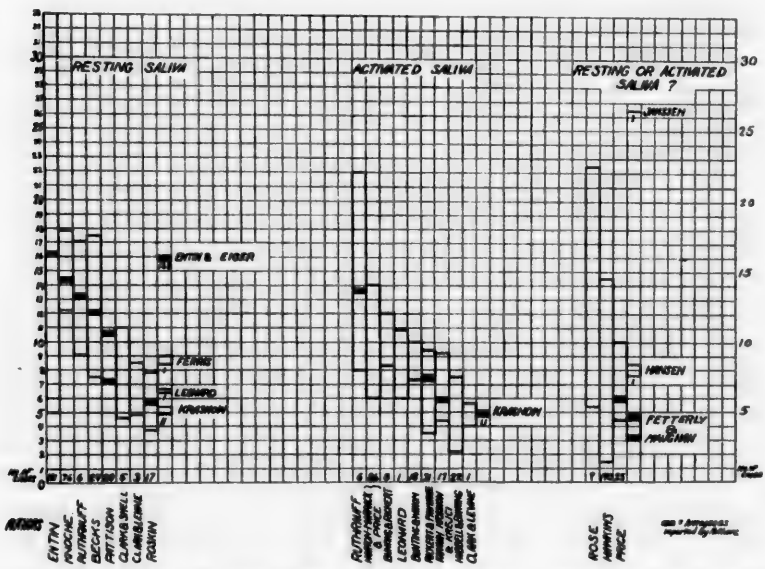

FIG. 1. Variations of results of former salivary analyses. Numerals along left and right sides indicate "mg. percent of Ca." Number of cases studied: indicated along bottom edge, immediately above names of authors. (The values obtained by Hawkins must be multiplied by 10; see footnote on p. 400.)

(1) that there is wide variation in the published results (a) as to range and (b) average; (2) variations in range and average seem to be due to differences in method and technic, as well as to selection of material; (3) no conclusions can be drawn as to difference in Ca composition of resting and activated saliva, for the values overlap throughout; (4) the results published by the authors in Group III (*table 3*), for resting saliva as well as activated saliva, tend to group themselves at the lower levels.

IV. APPLICATION OF THE FUNDAMENTALS OF Ca DETERMINATION TO ANALYSIS OF TEST SOLUTIONS AND SALIVA

A. Test solution. The great variation in results obtained by the different technics of former authors, in the determination of Ca in saliva, indicated special need to control the factors in the analytical procedure. Several methods were tested, of which the procedures of Halverson and Bergeim and of Kirk and Schmidt were found to be the most useful. Analyses were made of a test solution containing a known concentration of Ca, prepared by drying $CaCO_3$ (J. T. Baker's Special) in a vacuum desiccator for 18 hours; transferring 0.25022 gms. to a liter flask; adding 200 cc. of redistilled H_2O and 10 cc. of concentrated HCl; and, after solution was complete, diluting to the mark with H_2O. The samples were set up as follows: 2 cc. amounts of test solution were transferred to 15-cc., conical, pyrex centrifuge-tubes. To each was added 2 cc. of redistilled H_2O and, carefully, 1 cc. of 4 percent $(NH_4)_2C_2O_4$. The pH of the mixture was adjusted by adding 2 drops of 0.04 percent bromcresol green, and 1:1 NH_4OH as required—just to change the color of the indicator from yellow to greenish blue.

Results obtained with Van Slyke and Sendroy's modification of the Halverson and Bergeim method are recorded in *table 4*. Each result represents a single determination. The method gives consistent results, not only on 2-cc. amounts, which correspond to those in 2 cc. of blood serum, but also on smaller and larger amounts (1–5 cc.). Maximum variations from the theoretical value in 2-cc. samples were $+2.5$ and -2.1 percent, which fulfills the usual requirements for this method. On larger amounts, accuracy was greatly increased; on 1-cc. samples, results remained within the permissible error of ± 3 percent, but maximum variations were more frequent. These data prove that satisfactory results can be obtained with this method on test solutions, when amounts of Ca corresponding to those in 2 cc. of saliva are used. With Kirk and Schmidt's method, the results (*table 5*) were also within the permissible error of ± 3 percent, with one exception; and all but 4 of the 26 determinations were within ± 2 percent of the theoretical value. In other words, Ca can be satisfactorily recovered in amounts as small as 0.2 mg. by the method of Kirk and Schmidt. In a large number of tests with known amounts of Ca the results obtained by both methods indicated that they were accurate.

TABLE 4

Analyses of test solution containing 10.0 mg. percent of Ca, by Van Slyke and Sendroy's modification of the Halverson-Bergeim method

DATE	AMOUNT	Ca PER 100 CC. OF SOLUTION		DATE	AMOUNT	Ca PER 100 CC. OF SOLUTION	
		Found mg.	Deviation percent			Found mg.	Deviation percent
	cc.				cc.		
9/4	2	10.25	+2.5	9/11	1	9.70	−3.0
		9.95	−0.5			10.09	+0.9
9/5		10.0	0			10.09	+0.9
		10.0	0			9.9	−1.0
		9.85	−1.5		2	10.14	+1.4
		10.1	+1.0			9.85	−1.5
9/23		9.79	−2.1			9.99	−0.1
		9.89	−1.1			10.23	+2.3
		9.79	−2.1		4	10.12	+1.2
		10.09	+0.9			9.99	−0.1
						10.13	+1.3
						9.99	−0.1
					5	10.09	+0.9
						10.09	+0.9
						10.08	+0.8
						10.03	+0.3

TABLE 5

Analyses of test solution containing 10.0 mg. percent of Ca by Kirk and Schmidt's method

	DATE					
	9/15	9/17	9/26	9/28	10/8	10/11
Amount (cc.)	2	2	2	2	2	2
Ca per 100 cc. of solution:						
Milligrams	9.90	10.19	9.70	10.15	9.94	10.09
	9.85	10.21	9.85	10.00	9.80	9.94
	9.80	10.19	10.22	9.95	9.91	10.09
	9.90	10.19	10.09	10.49	10.20	10.11
	9.90					
	10.50					
Deviation (%)	−1.10	+1.90	−3.00	+1.50	−0.60	+0.90
	−1.50	+2.10	−1.50	±0.00	−2.00	−0.60
	−2.00	+1.90	+2.20	−0.50	−0.90	+0.90
	−1.00	+1.90	+0.90	+4.90	+2.00	+1.10
	−1.00					
	+0.50					

B. Saliva. In salivary analysis the following factors may influence the results: (*1*) *Amount of saliva.*—The methods of Halverson and Bergeim, and Kirk and Schmidt (*tables 4* and *5*), are accurate for about 0.2 mg. of Ca. This corresponds to the amount found in 2 cc. of normal blood-serum, and is approximately the same as that in 2 cc. of saliva. Occasional low amounts of Ca in saliva can be determined accurately, because Halverson and Bergeim's method gives satisfactory results on amounts as low as 0.1 mg., and permits routine use of 2-cc. amounts of saliva for a single determination. A larger amount, however, increases a method's accuracy, but also decreases its utility. For instance, a method requiring 10 cc. or more cannot be used in routine examination of a large number of cases, because many patients are unable to secrete this amount of resting saliva within a reasonable period; its use would hardly be feasible in analysis of blood serum.

(*2*) *Preliminary treatment* must include removal of organic matter, cells, and débris by means of centrifugation and deproteinization. The dry-ashing procedure, very effective when properly followed, presents obstacles because of the required time, care, and expense. The difficulties are greatly increased when more than a few samples are run at a time. Halverson and Bergeim simplified the procedure for blood serum by substituting deproteinization. Horton, Marrack and Price, and Karshan, Krasnow, and Krejci, made actual tests of its value for saliva. Horton, Marrack and Price precipitated mucin "as far as possible" by adding a drop of 10 percent acetic acid, shaking for one-half hour, and filtering through ash-free paper. To check the influence of possible loss of Ca with the precipitated mucin, and of contamination of the calcium-oxalate precipitate by organic matter, 27 samples were ashed without removal of mucin. The residue was dissolved in dilute HCl, neutralized with methyl red, and the analysis continued according to Clark. They reported satisfactory recovery of Ca in deproteinized saliva as compared to ashed saliva. Karshan, Krasnow and Krejci, comparing dry ashing and deproteinization on 27 samples, obtained equivalent results for Ca in each specimen. They deproteinized with trichloracetic acid (final concentration in the mixture was 6 percent), and removed the precipitate by centrifuging. Trichloracetic seems preferable to acetic acid (Horton, Marrack, and

Price) in view of its general application as a protein precipitant in biochemical analysis. It is apparent from these comparative tests that, in deproteinized saliva, Ca can be successfully precipitated, and the oxalate titrated, despite any remaining organic matter. This technic has a definite advantage over the ashing procedure.

In order to see the effect of differences in preliminary treatment, several tests on saliva were made by Halverson and Bergeim's method with ***preliminary centrifugation and no deproteinization*** (Kramer-Tisdall modification), as formerly used by Becks, and compared with those obtained by a modification of the Kirk-Schmidt method in which there

TABLE 6

Ca analysis of saliva by modified Halverson-Bergeim and Kirk-Schmidt methods

	PATIENT									
	370	369	243	168	246	174	175	18	176	178
Date	8/22	8/22	9/8	9/17	9/17	9/26	9/28	10/3	10/3	10/4
Method (mg. % Ca):										
Halverson-Bergeim[1]	6.8	5.92	3.8	4.175	4.85	6.30	5.65	7.10	5.96	5.40
	6.8	6.02	3.9	4.175	4.75	6.35	....	7.10	6.01	5.15
						6.21	5.65	7.30	6.07	5.15
						6.25	5.75	7.05	6.07	5.45
Kirk-Schmidt[2]	7.29	6.31	4.65	4.950	4.95	6.80	6.22	8.64	6.69	5.85
	7.29	6.60	4.57	5.140	5.24	6.80	5.94	8.59	6.64	6.00
						6.80	....	8.23	6.58	6.31
						6.70	5.26	8.23	6.74	5.90

[1] Preliminary centrifugation and no deproteinization.

[2] Deproteinization and no preliminary centrifugation.

was ***deproteinization*** but ***no preliminary centrifugation***, the latter treatment corresponding to that used by most of the authors in Group III. This series of comparative values on nine samples of saliva is shown in *table 6*. It is interesting to observe that the results do not agree, the values obtained when deproteinization with no preliminary centrifugation was used being higher in every instance. Since our analyses of test solution show that the precipitation and washing procedures worked satisfactorily, these differences are evidently due to the dissimilar preliminary treatments. This influence was determined in another series of tests (*table 7*).

The original method of Halverson and Bergeim calls for preliminary

centrifugation (because it was applied to serum) and deproteinization, whereas Kirk and Schmidt's method calls for preliminary centrifugation *without* deproteinization.[6] If we compare the values obtained, on the same sample of saliva, with these two methods (*table 7*), we find that the results with the Halverson-Bergeim method (2A)[7] ranged between 0.28 and 0.83 mg. percent higher than those of Kirk and Schmidt's method (1B). This difference must be due to presence of protein at the time of Ca precipitation by the Kirk-Schmidt method, this protein,

TABLE 7

Influence of differences in preliminary treatment of saliva on results of Ca analysis

	SAMPLE OF SALIVA							
	I		II		III		IV	
Method	A H. & B.	B K. & S.	A H. & B.	B K. & S.	A H. & B.	B K. & S.	A H. & B.	B K. & S.
1—Prelim. centrif. No deproteinization	3.66	3.37[2]	3.52	3.15[2]	3.64	3.64[2]	3.64	3.04[2]
	3.66	3.38[2]	3.63	3.66[2]	3.44	3.54[2]	3.64	3.34[2]
2—Prelim. centrif. Deproteinization	4.02[1]	4.04	4.05[1]	3.88	3.92[1]	4.11	4.07[1]	3.98
	4.02[1]	4.04	4.05[1]	3.88	3.82[1]	4.21	3.97[1]	3.98
3—No prelim. centrif. Deproteinization	4.44	4.30	4.25	4.60	4.11	4.41	4.07	4.18
	4.33	4.30	4.20	4.60	4.11	4.31	4.07	4.48
4—No prelim. centrif. No deproteinization	15.00	3.21	13.80	....	8.00	3.44	6.00	3.24
	9.00	3.26	7.50	....	9.00	3.54	7.00	3.44

[1] Results obtained with original Halverson-Bergeim method (modification of Van Slyke and Sendroy).

[2] Results obtained with original Kirk-Schmidt method.

holding some Ca in solution, being lost on washing, with ensuing lower values. This finding is further confirmed by results with a modification of the Halverson-Bergeim method in which deproteinization was omitted, these values (1A), when compared with 2A (original Halver-

[6] Checks with the Halverson-Bergeim method varied within a narrower range than those obtained by the original Kirk-Schmidt method. This may be due to the fact that the Halverson-Bergeim method has been used for routine analysis in this laboratory for several years, and worked more efficiently in our hands than the more recent method of Kirk and Schmidt.

[7] The Arabic numerals refer to the procedures indicated at the left margin.

son-Bergeim method), being from 0.33 to 0.47 mg. percent lower. Loss of Ca was confirmed by another test with the Kirk-Schmidt method, modified by adding deproteinization just after preliminary centrifugation (2B). The non-deproteinized samples (1B) yielded results falling from 0.48 to 0.79 mg. percent below those for 2B. If the two factors of preliminary centrifugation and deproteinization are kept constant in the Halverson-Bergeim, and Kirk-Schmidt, methods (2A and 2B), the results check very well. However, in parallel tests in which we did *not* deproteinize in the Halverson-Bergeim, and Kirk-Schmidt, methods, check-results were less satisfactory. In each case the values under 1A were found to be higher than under 1B. We suppose this difference is due to use of filters for washing, in the Kirk-Schmidt method (which may have held a certain amount of organic material); whereas, in the Halverson-Bergeim method, organic material may have gone down with the calcium-oxalate precipitate and, at the time of titration, neutralized permanganate, causing higher values. In a further series (3 A and B), the samples were not centrifuged as a preliminary procedure. For both methods, these values ran higher than those of series 1 and 2. The values under 3A and 3B again "gave checks" by the two methods. It has been emphasized before that preliminary centrifugation seems to be essential to eliminate foreign bodies and cells, which influence the results. But, in the process of centrifugation, some Ca may be carried down, thereby also influencing the results. These influences are tested in a comparison of series 2 and 3. The values of 2 are slightly lower than those of 3, suggesting that Ca was lost by preliminary centrifugation—a loss ranging in these four sets of analyses between 1 and 16 percent. On the other hand, the values for series 3 may be too high because Ca was derived from cells or débris. In a final series, both centrifugation and deproteinization were omitted (4A and 4B). Presence of organic matter, cells, and débris, led with the Halverson-Bergeim method (4A) to greatly increased values, because this material neutralized much permanganate in titration. With the Kirk-Schmidt method (4B), we obtained the lowest values for any modification. This must be explained as for the values under 1B; namely, that the organic matter held large amounts of Ca in solution at the time of precipitation, the organic matter being largely eliminated by the filters during the washing.

In evaluating the experience gained from these four series of tests on saliva (*table 7*), serious consideration must be given, in the final choice of method, to two important questions: Is it necessary to deproteinize, and to use preliminary centrifugation, to come closest to the actual Ca content? From *table 7*, it is evident that many factors affect the procedure in series 1 and 3, 1 giving results which are evidently too low; 3, results which are apparently too high. Of course, all results in series 1, 2 and 3—the values in series 4 cannot be considered at all—are more or less relative, because unknown factors influenced them. Results obtained by the original methods of Halverson and Bergeim, and Kirk and Schmidt, cannot be compared with each other when applied to saliva because of the difference in preliminary treatment. We believe that the *original* Halverson-Bergeim method (2A) and the *modified* Kirk-Schmidt method (2B) are preferable.

V. SUMMARY AND CONCLUSIONS

Becks pointed out (2) that standard values for electrolytes in mixed saliva must be established, if oral diseases are ever to be correlated with changes in its composition. The number of analyses up to 1929 was very small, and only a few have since been published. Even though current literature is filled with theoretical explanations of ways in which saliva, through its inorganic constituents, may influence the health of dental structures, little attention has been given to actual analytical work along these lines. We consider it of utmost importance that standard values for mixed saliva be established for the same reason that it was necessary by long and arduous work to establish standard values for blood serum. We know that deviations from normal levels of serum are indicative of disturbances. Rickets, tetany, Paget's disease—a number of endocrine or constitutional disturbances, etc.—induce definite changes in the composition of blood serum.

In former publications on salivary studies, the authors usually had preconceived ideas as to the solution of their problems; i.e., they *wanted to find* a correlation between faulty composition of saliva and dental diseases. However, most of the authors overlooked very important facts: (1) No standard values for normal composition of mixed saliva have ever been established. (2) In examining cases with

pathologic lesions of the oral cavity (caries, paradentosis, etc.), little or no attention has been paid to the general physical condition of the patient. (3) Cases immune to caries were recorded as normal, and analytic results for their saliva were used as standards for comparison with pathologic cases; but immunity to caries is not always an indicator of health. In paradentosis, for instance, especially in the "dystrophic type," very frequently there is antagonism to caries; i.e., no decay. Some authors called such patients normal. But the dystrophic type of paradentosis very frequently has an etiological background of metabolic disturbances, which may definitely affect salivary composition. (4) Since most authors had preconceived ideas, no attempt was made to find out whether definite changes in salivary composition were *results*, as distinct from *causes*, of pathologic lesions in the oral cavity. All analyses of saliva in previous years, in which the above-mentioned factors were disregarded, are therefore of very little practical value. It is not surprising that, at the end of most investigations, the authors were unable to correlate or interpret their findings. It must be realized, more and more clearly in the future, that the etiology of outstanding dental disturbances cannot be determined unless modern aids in biological investigation are fully utilized. Biochemistry will probably take a leading part, but only by employment of generally accepted methods which have been thoroughly checked for errors. Only persons with adequate training in biochemical methods should engage in this phase of the work.

In an attempt to evaluate and compare the work of former investigators as to their technical procedures, selection of material, and results of Ca analysis, the publications of twenty-six authors were available. With very few exceptions all used unlike technical procedures; there was no uniformity as to the general health of the patients. The results published by eight of these authors (Group I, *table 3*) cannot be used for the establishment of normal standard values because the methods were inadequate and unsuitable. There is no agreement between their results and those obtained by more modern methods. Eight others (Group II) incompletely reported their procedure, making it impossible to learn whether they paid attention to important steps in the preliminary treatment of saliva, selection of material, etc. The fact that they referred to the technic as having

been suggested by an originator of a Ca method does not mean that the requirements of salivary analysis were fulfilled. The results given by several authors range from 3.2 to 33 mg. percent of Ca. Since valuable data from which the reliability of the procedures can be judged are missing, the work of the authors in Group II is of little value for comparison with that of the ten authors who completely reported their data (Group III). From the reports of these ten authors, it is evident that a number of factors in their procedures modified the results for Ca distribution. From a strictly scientific standpoint, only the analyses of Clark and Shell seem to be reliable and can be used for the establishment of normal standard values. With certain reservations, the results of Leonard, Becks, and Krasnow can be thus utilized, but each failed in one or more respects to pay attention to essential factors. This means that, of the hundreds of analyses that have been made, data for less than fifty persons free from metabolic disturbances are available for the establishment of normal standards. This condition suggests the reason why we lack definite knowledge of the biochemical relationships between dental structures and the fluids that bathe them.

It is felt that the failure of former investigators is due not only to the slow development of analytical science, but also to lack of biochemical training of most authors who worked in this field. The problem of salivary studies is extremely complex. An analysis of the work done heretofore was necessary, to provide a definite basis for future analyses; to stimulate interest in this field; and to outline a procedure which, based on accepted principles, would give satisfactory results and also throw light upon the true conditions of Ca distribution in saliva. The most important phases to be considered in the establishment of normal standards are selection of material and analytical procedure. In the *selection of material*, special attention must be paid to the health of the individual whose saliva is to be analyzed. He should be examined physically, with special attention to disturbances of the endocrine system and in the mineral metabolism. Patients must be questioned as to their nutrition. Knowledge of dental conditions is necessary to detect pathologic processes that may influence salivary composition. Age must be considered in recording the data, because of changes that may occur during the different periods of life. Reported differences

between activated and resting salivas, and influences of different stimuli during activation, suggest that only resting saliva be utilized. It is important to record rate of flow, which varies among different individuals, and at different times in the same individual.

In the *analytical procedure* for Ca studies, it is necessary to use methods accepted in biochemical science, and to adapt fundamental principles relating to blood serum and other body fluids to the special requirements of saliva. In most instances, former investigators made up their own methods, disregarding one or more very important matters that unfavorably influenced their results. Two main steps in the analytical procedure usually occasioned errors: preliminary treatment of saliva, and technic of washing precipitated Ca. *Preliminary treatment* embraces two major considerations that are frequently ignored: Shall the saliva be centrifuged preliminary to precipitation of Ca? Shall the saliva be deproteinized to free it from organic matter that interferes with accuracy? Preliminary centrifugation, serving to remove cellular elements, débris, and other foreign substances, was disregarded by nine of the ten investigators who completely reported their procedures (Group III, *table 1*). This procedure is necessary, despite possible loss of Ca—a loss that should be negligible and of less serious consequence than the high values resulting from presence of débris, etc. The loss of 6–18 percent, as reported by Clark and Levine, was partially confirmed by us in four analyses, in which it ranged from 1 to 16 percent, and averaged about 6. It is possible that this slight loss is due to preliminary removal of débris instead of to centrifugation. Results obtained by authors who have not used preliminary centrifugation must, therefore, tend to be high. These facts are illustrated by our own experiments (*table 7*, series 1 to 4). Removal of organic substances from saliva has been considered by most former investigators, who used either an ashing procedure or deproteinization. This step enabled them completely to precipitate Ca. When deproteinization, the modern way of removing interfering organic substances, was used with no preliminary centrifugation—a procedure followed by most authors—higher results were obtained than when preliminary centrifuging was followed by deproteinization (*table 7*, series 3 and 4). The highest results were obtained when neither deproteinization nor centrifugation was used as a preliminary

treatment. When centrifugation was used without deproteinization, the lowest of all values were obtained with the Halverson-Bergeim method (*table 7*, series 1 and 2). Preliminary treatment of saliva in future Ca analyses should, therefore, include centrifugation—and removal of organic matter by ashing or more convenient deproteinization.

The *technic of washing* precipitated Ca has given rise to improvements of the Halverson-Bergeim method, to permit analysis of smaller amounts of material. This led finally to modifications suggested by Van Slyke and Sendroy, Clark and Collip, Stanford and Wheatley, and Kirk and Schmidt, who found that their own improvements gave in their hands, most satisfactory results, when tested on known amounts of Ca. It is generally accepted that any of these washing technics is satisfactory, if the analysts have had the necessary experience. The main question to be considered in the washing technic is whether to decant, siphon, or filter, the usefulness of each varying with the analyst, and must be determined on a test solution. In our hands, the filtration method of Kirk and Schmidt, which seems to avoid a number of possible errors in the centrifuge technic, has worked very satisfactorily and is, therefore, discussed in detail in this paper as adapted to saliva. For washing, it is necessary to use a saturated solution of calcium oxalate in dilute ammonia, because the required volume of wash liquid is large. In the Halverson-Bergeim centrifuge-technic, the wash liquid may be either plain dilute ammonia, or that saturated with calcium oxalate. The saturated solution permits use of a larger volume of liquid to wash the tube and remove interfering ammonium oxalate. A comparatively small amount of plain dilute ammonia may also be used. In this case, loss of precipitated calcium oxalate by solution in the wash liquid is compensated by a minute amount of residual ammonium oxalate in the tube after washing. After centrifuging, the wash liquid may be removed in one of two ways: by decanting (Clark-Collip), which involves the possibility of losing a few calcium oxalate crystals but, when properly carried out, is the simplest procedure; or by siphoning with a pipette having an upturned tip (Halverson and Bergeim), to avoid disturbance of the precipitate. The latter method, in conjunction with the comparatively small volume of plain, dilute ammonia (the modification of Van Slyke and

Sendroy), has given the most uniform results in our hands. From the standpoint of washing technic, therefore, the procedures of Halverson and Bergeim, and of Kirk and Schmidt, are satisfactory.

So far as Ca level in saliva is concerned, very little progress has been made since Röse (1905) published the first long series of results. It is intended, in a further investigation of Ca distribution in saliva, to apply properly controlled methods, and to establish normal standard values as a basis for future work. (The second paper in this series will follow in an early issue—*Ed.*)

BIBLIOGRAPHY

(1) Agnew, M. C., Agnew, R. G., and Tisdall, F. F. The production and prevention of dental caries, *J. A. D. A.*, *20*, 193, 1933.

(2) Becks, H. Ergebnisse neuerer Speichel-Untersuchungen mit besonderer Berücksichtigung der Gravidität und der Paradentosen; Meusser, Berlin, 1929. (3) Electrolytes of saliva under normal and pathological conditions, *Proc. Soc. Exp. Biol. and Med.*, *26*, 93, 1928.

(4) Bell, B. Cited by Broderick, F. W. (6).

(5) Briggs, A. P. Some applications of the colorimetric phosphate method, *J. Biol. Chem.*, *59*, 255, 1924.

(6) Broderick, F. W. The effect of endocrine derangement on the teeth, *Brit. Dent. J.*, *41*, 955, 1920. (7) Dental medicine; Mosby Co., St. Louis, 1928.

(8) Bunting, R. W., and Rickert, U. G. Dental caries, *J. Nat. Dent. Assn.*, *2*, 247, 1915.

(9) Bunting, R. W., and Wixon, F. H. Dental caries, *Ibid.*, *4*, 81, 1917.

(10) Clark, E. P., and Collip, J. B. A study of the Tisdall method for the determination of blood serum calcium with a suggested modification, *J. Biol. Chem.*, *63*, 461, 1925.

(11) Clark, G. W. The microdetermination of calcium in whole blood, plasma, and serum by direct precipitation, *Ibid.*, *49*, 487, 1921. (12) With Levine L. Inorganic constituents of human saliva: II, *Amer. J. Physiol.*, *81*, 264, 1927. (13) With Shell, J. S. The influence of diet upon the inorganic constituents of human saliva, *Den. Cosmos*, *69*, 500, 605, 1927.

(14) De Waard, D. J. A direct micro-estimation of calcium in the serum, *Biochem. Zeit.*, *97*, 186, 1919.

(15) Entin, D. A. Bedeutung des gemichten Speichels in der Physiopathologie der Mundhöhle, *Deut. Monat. f. Zahnh.*, *45*, 930, 1927. (15A) Zur physikalisch-chemischen Theorie der Karies, *Zeit. f. Stomat.*, *27*, 239, 1929.

(16) Ferris, H. C. Mouth hygiene, controlled by diet, through salivary analysis, *Den. Cosmos*, *62*, 453, 1920. (17) With Smith, E. E., and Graves, E. V. Physiological and pathological variations in the composition of human saliva, *J. A. D. A.*, *10*, 19, 1923.

(18) Fetterly, M., and Maughan, G. H. The stability of calcium in the saliva and blood, *Amer. J. Hyg.*, *14*, 723, 1931.

(19) FISKE, C. H., AND ADAMS, E. T. Determination of calcium by alkalimetric titration, *J. Amer. Chem. Soc.*, *53: 2*, 2498, 1931.

(19A) FRERICHS. Cited by Matthews: Physiological Chemistry, 322; *3rd ed.*, 1921: Wm. Wood & Co., New York.

(20) GREENWALD, I. The estimation of non-protein nitrogen in blood, *J. Biol. Chem.*, *21*, 61, 1915.

(21) HALVERSON, J. O., AND BERGEIM, O. The determination of small amounts of calcium, particularly in blood, *Ibid.*, *32*, 159, 1917.

(22) HAMILTON, B. A comparison of the concentrations of inorganic substances in serum and spinal fluid, *Ibid.*, *65*, 101, 1925.

(23) HAMMERBACHER. Quantitative Verhältnisse der org. und anorg. Bestandteile des menschl. gemischten Speichels, *Zeit. f. physiol. Chem.*, *5*, 302, 1881.

(24) HAMMARSTEN. Lehrbuch der Physiologischen Chemie, 356; 1922.

(25) HANSSEN, R. Zur Calciumbestimmung im Speichel, *Deut. Monat. f. Zahnh.*, *51*, 603, 1933.

(26) HAWKINS, H. F. (26) With HENDRICKS, W. W., AND HILLS, N. R. The cause and prevention of dental decay as indicated by a survey, *J. A. D. A.*, *15*, 354, 1928. (27) Dental decay: what it is, and means for its control, *Ibid.*, *16*, 781, 1929. (28) The cause and control of caries and pyorrhea, *Proc. Pac. Coast Dental Conference*, *2*, 257, 1929. (29) A rational technique for the control of caries and systemic pyorrhea, *J. Den. Res.*, *11*, 201, 1931. (30) What is the cause of caries and systemic pyorrhea?, *J. A. D. A.*, *18*, 943, 1931.

(31) HORTON, K., MARRACK, J., AND PRICE, I. The relation of calcium in the saliva to dental caries, *Biochem. J.*, *23*, 1075, 1929.

(32) HUBBELL, R. B., AND BUNTING, R. W. Calcium and phosphorus of saliva in relation to dental caries, *J. Nutrition*, *5*, 599, 1932.

(33) INOUYE, J. M. Biochemical studies of salivary mucin, *J. Den. Res.*, *10*, 7, 1930.

(34) JACOBI, F. Über die quantitative Zusammensetzung der Asche vom gemischten menschl. Speichel, Diss., 21; Würzburg, 1896.

(35) TÜRKHEIM, TECKLENBURG, LICHTENSTEIN, PRELLE, AND JANSSEN. Über den Gehalt des Speichels an Mucin, Ammoniak, Kochsalz, und Calcium, *Deut. Monat. f. Zahnh.*, *44*, 897, 1926.

(36) JANSSEN, J. Über den Kalziumgehalt des Speichels und seine Beziehung zur Zahnkaries, Diss.; Hamburg, 1925.

(36A) JANSEN, W. H. Ca in man: I. Estimation of Ca in blood, *Zeit. f. physiol. Chemie.*, *101*, 176, 1918.

(37) KARSHAN, M., KRASNOW, F., AND KREJCI, L. E. A study of blood and saliva in relation to immunity and susceptibility to dental caries, *J. Den. Res.*, *11*, 573, 1931.

(38) KIRK, P. L. (38) With SCHMIDT, C. L. A. An improved technique for microcalcium analysis, *J. Biol. Chem.*, *83*, 311, 1929. (39) With MOBERG, E. G. Microdetermination of calcium in sea water, *Ind. and Eng. Chem.*, *5*, 95, 1933.

(40) KNOCHE. Ueber den Ionengehalt des Speichels und seine Beziehungen zur Zahnkaries. *D. Zahn. Woch.*, *30*, 419, 1927.

(41) KRAMER, B., AND TISDALL, F. F. A simple technique for the determination of calcium and magnesium in small amounts of serum, *J. Biol. Chem.*, *47*, 475, 1921. (42) The direct quantitative determination of sodium, potassium, calcium and magnesium in small amounts of blood, *Ibid.*, *48*, 223, 1921.

(43) KRASNOW, F. Biochemical studies of dental caries: diurnal variations in the P, Ca, H-ion concentration, and titratable alkalinity, *J. Den. Res.*, *12*, 530, 1932. (43A) With KARSHAN, M., AND KREJCI, L. E. The determination of calcium and phosphorus in saliva, *J. Lab. and Clin. Med.*, *17*, 1148, 1932.

(44) KUTTNER, T., AND COHEN, H. R. Micro-colorimetric studies: I. A molybdic acid stannous chloride reagent; the micro-estimation of phosphate and calcium in pus, plasma, and spinal fluid, *J. Biol. Chem.*, *75*, 517, 1927.

(45) LAIDLAW, P. P., AND PAYNE, W. W. A method for the estimation of small quantities of calcium, *Biochem. J.*, *16*, 494, 1922.

(46) LEONARD, H. J. The causes of variation in salivary calcium; their relation to susceptibility and immunity to dental caries, *J. A. D. A.*, *15*, 1530, 1928.

(47) LYMAN, H. A rapid method for determining Ca in blood and milk, *J. Biol. Chem.*, *29*, 169, 1917.

(48) KLEIN, H., AND MCCOLLUM, E. V. A preliminary note on the significance of the phosphorus intake in the diet and blood phosphorus concentration in the experimental production of caries-immunity and caries-susceptibility in the rat, *Science*, *74*, 662, 1931.

(49) MCCRUDDEN, F. H. The quantitative separation of calcium and magnesium in the presence of phosphates and small amounts of iron, devised especially for the analysis of foods, urine, and feces, *J. Biol. Chem.*, *7*, 83, 1909–10. (50) The determination of calcium in the presence of magnesium and phosphates: the determination of calcium in urine, *Ibid.*, *10*, 187, 1911–12.

(51) PATTISON, C. L. Dietetic conditions which influence the calcium content of saliva, *Brit. Med. J.*, *2*, 6, 1926.

(52) PETERS, J. P., AND VAN SLYKE, D. D. Quantitative clinical chemistry; Vol. II: Methods; Williams and Wilkins, Baltimore, 1932.

(53) PICKERILL, H. P. The prevention of dental caries and oral sepsis; 2nd ed., 1914; S. S. White Dental Manufacturing Co., Philadelphia.

(54) PRIBRAM, R. Cited by Clark, G. W. (11).

(55) PRICE, W. A. Calcium metabolism studies on the nature and rôle of the activators: researches on fundamentals for the prevention of dental disease, *J. A. D. A.*, *16*, 265–287, 1929. (56) Control of dental caries and some associated degenerative processes through reinforcement of the diet with special activators, *Ibid.*, *19*, 1339, 1932. (57) The experimental basis for a new theory of dental caries, with chemical procedures for determining immunity and susceptibility, *Den. Cosmos*, *74*, 1139, 1932. (58) Why dental caries with modern civilization?: Part V, *Den. Digest*, *39*, 266, 277, 1933. (59) Additional light on the etiology and nutritional control of dental caries with its application to each district showing immunity and susceptibility, *J. A. D. A.*, *20*, 1648, 1933.

(60) RICKERT, U. G., AND PALMERLEE, F. A comparative study of total salivary and blood calcium in varying types of individuals, with special reference to cases of pregnancy, *Ibid.*, *11*, 783, 1924.

(61) ROE, J. H., AND KAHN, B. S. A colorimetric method for the estimation of blood calcium, *J. Biol. Chem.*, *67*, 585, 1926. (62) A colorimetric determination of blood calcium, *Ibid.*, *81*, 1, 1929.

(63) RÖSE, C. Zahnverderbnis und Speichelbeschaffenheit, *Deut. Monat. f. Zahnh.*, *23*, 705, 1905.

(64) ROSKIN, M. Mineral content of saliva of children with arrested dental caries, *Proc. Soc. Exp. Biol. and Med., 25*, 465, 1927–28.

(65) RUTHRAUFF, W. M. Landmarks in dental research leading to the establishment of the calcification theory of immunity to dental caries; W. M. Ruthrauff Co., Philadelphia, 1923.

(66) SHOHL, A. T. The effect of hydrogen-ion concentration upon the determination of calcium, *J. Biol. Chem., 50*, 527, 1922.

(67) SPENCER-PAYNE. Saliva: its protective action upon the teeth, *Brit. Dent. J., 45*, 1637, 1924.

(68) STANFORD, R. V., AND WHEATLEY, A. H. M. The estimation of calcium in blood, *Biochem. J., 19*, 710, 1925.

(69) TINGEY, A. H. The determination of Ca in blood-serum and cerebrospinal fluid, *Ibid., 27*, 332, 1933.

(69A) TISDALL, F. F. A note on the Kramer-Tisdall method for the determination of calcium in small amounts of serum, *J. Biol. Chem., 59*, 439, 1923.

(70) TREVAN, J. W., AND BAINBRIDGE, H. W. Estimation of calcium in blood serum, *Biochem. J., 20*, 423, 1926.

(71) TÜRKHEIM. Die Ernahrung und die physikalische Chemie des Schmelzes, *Vierteljs. f. Zahnh., 38*, 487, 1922. (71A) Die Wasserstoffionenkonz. d. Speichel, *Deut. Monat. f. Zahnh., 43*, 44, 1925.

(72) VAN SLYKE, D. D., AND SENDROY, J., JR. Gasometric determination of oxalic acid and calcium, and its application to serum analysis, *J. Biol. Chem., 84*, 217, 1929.

(73) Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists; *2d ed.*, 1923; Washington, D. C.

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

Organized: New York City, Dec. 10, 1920. Of those at the meeting of organization, the following remain active members: Adolph Berger, Theodor Blum, G. W. Clapp, H. S. Dunning, W. J. Gies, H. W. Gillett, R. G. Hutchinson, Jr., T. P. Hyatt, Milo Hellman, F. C. Kemple, A. H. Merritt, B. B. Palmer, P. R. Stillman, L. M. Waugh.

General Meetings have been held on this schedule: (1) New York, Dec. 21, '22. (2) Chicago, Mar. 7, '24. (3) Chicago, Mar. 20, '25. (4) Chicago, Mar. 26, '26. (5) New York, Apr. 19, '27. (6) Washington, Mar. 25–28, '28. (7) Chicago, Mar. 23–24, '29. (8) Toronto, Mar. 24–26, '30. (9) Memphis, Mar. 21–22, '31. (10) Columbus, Mar. 19–20, '32. (11) Chicago, Mar. 18–19, '33; adjourned executive session, New York, May 25, '33. (12) Chicago, Mar. 17–18, '34. *Next general meeting* (13): Chicago, Mar. 16–17, in coördination with the twelfth annual meeting of the American Association of Dental Schools, Mar. 18–20, 1935.

Objects (Const., Art. II): . . . "(a) To promote broadly the advancement of active research in all branches of dental science and in related phases of the sciences that contribute directly to the development of dentistry, and which add to the knowledge of the mouth and teeth and of their relations to the body as a whole; . . . (b) to encourage and facilitate coöperative effort and achievement by, and mutual helpfulness among, investigators in all nations in every division of stomatology; (c) to the end, particularly, that dentistry may render cumulatively more perfect service to humanity." (The Association has been using "dentistry" and "stomatology" as synonyms.)

Membership: total (June 15), 360. *Eligibility (Const., Art. 14, Sec. 1).*—"Any person who has conducted, and published an account of, a meritorious original investigation in dental science, or in any of the sciences contributory to stomatology, shall be eligible to membership in this Association, providing such person conforms to the recognized standards of professional ethics." *Election.*—New members may be elected *only at general meetings of the Association.* Membership in a section is secondary to membership in the Association as a whole. Removal of members from one geographic center to another does not affect their membership in the Association.

Endowment fund (as of April 21, 1934): $1,119.23. There have been no expenditures from this fund.

Divisions may be organized by the members in any nation. There are now divisions in Austria, Canada, China, Czechoslovakia, England, Hungary, South Africa, and the United States—8. *Sections* may be organized by the members in geographic centers within the divisions. There are now sections (26) in Vienna ('29); Halifax ('28), Toronto ('21), Winnipeg ('30); Chengtu ('34); Prague ('32); London ('31); Budapest ('34); Johannesburg ('34); Ann Arbor ('23), Baltimore ('33), Boston ('20), Chicago ('20), Cleveland ('30), Columbus ('32), Louisville ('32), Minnesota ('28), New Haven ('30), New York ('20), Philadelphia ('28), Pittsburgh ('28), Richmond ('33), Rochester ('33), St. Louis ('28), San Francisco ('24), Washington, D. C. ('31). The names of the secretaries are given on p. 2 of this cover.

Sectional meetings in 1933–34 (schedule subject to revision). Ann Arbor—May 8. *Baltimore*—Oct. 24, Nov. 15, Jan. 31. *Boston*—Nov. 6, Jan. 29, May 21. *Chicago*—Oct. 27, Jan. 26, May 18. *Cleveland*—Feb. 12. *Columbus*—Mar. 9. *Halifax*—Nov. 19, Jan. 25, Mar. 14. *London*—Oct. 18, Dec. 6, Jan. 24, Apr. 18. *Louisville*—May 11. *Minnesota*—Nov. 20, Jan. 22, Feb. 26. *New York*—Dec. 7, June 7. *Philadelphia*—Oct. 13. *Pittsburgh*—Oct. 2, Nov. 6, Dec. 4, Jan. 8, Mar. 19, May 7. *Prague*—Jan. 12, May ?. *Richmond*—Nov. 18, Apr. 18, May 26. *Rochester*—Oct. 13, 27; Nov. 10, 24; Dec. 8, 22; Jan. 5, 19; Feb. 2, 16; Mar. 2, 16; Apr. 13, 27; May 11, 25. *St. Louis*—Nov. 14, Jan. 9, Apr. 10. *San Francisco*—Nov. 16, Jan. 24, Feb. 28, Mar. 28, Apr. 25. *Toronto*—July 17, Sept. 12, Oct. 20, Jan. 18. *Washington*—May 3. *Winnipeg*—Feb. 7, May 28.

Journal of Dental Research: now the Association's official publication. See register of officers and editors on page 2 of this cover.

JOURNAL OF DENTAL RESEARCH

HONORARY EDITORS WHO ARE ABOUT TO RETIRE WITH THE ORIGINAL MANAGEMENT

THEODORE B. BEUST
Louisville
ARTHUR D. BLACK
Evanston
JAMES R. BLAYNEY
Chicago
C. F. BÖDECKER
New York City
B. HOLLY BROADBENT
Cleveland
R. W. BUNTING
Ann Arbor
FRANK COLYER
London
EDGAR D. COOLIDGE
Chicago
KAETHE W. DEWEY
Pittsburgh
F. N. DOUBLEDAY
London
WM. G. DOWNS, JR.
Chicago
C. L. DRAIN
Iowa City
WM. B. DUNNING
New York City
J. J. ENRIGHT
Pittsburgh
GUIDO FISCHER
Hamburg
H. E. FRIESELL
Pittsburgh
HENRY W. GILLETT
New York City
SAMUEL M. GORDON
Chicago
BERNHARD GOTTLIEB
Vienna
WILLIAM K. GREGORY
New York City
EDWARD H. HATTON
Chicago
MILO HELLMAN
New York City
THOMAS J. HILL
Cleveland
PERCY R. HOWE
Boston
THADDEUS P. HYATT
New York City
PHILIP JAY
Ann Arbor
JAN JESENSKY
Prague
V. H. KAZANJIAN
Boston
HENRY KLEIN
New Haven
RUDOLF KRONFELD
Chicago
W. H. G. LOGAN
Chicago
OTTO LOOS
Frankfort
E. V. MCCOLLUM
Baltimore
FREDERICK S. MCKAY
New York City
JOHN A. MARSHALL
San Francisco
ARTHUR H. MERRITT
New York City
C. T. MESSNER
Washington
L. M. S. MINER
Boston
FREDERICK B. NOYES
Chicago
BALINT ORBAN
Vienna
BISSELL B. PALMER
New York City
WESTON A. PRICE
Cleveland
F. M. PUCCI
Montevideo
ISAAC SCHOUR
Chicago
WM. G. SKILLEN
Chicago
WILMER SOUDER
Washington
JOSEF SZABO
Budapest
KURT H. THOMA
Boston
M. L. WARD
Ann Arbor
L. M. WAUGH
New York City
WM. H. WELKER
Chicago
WALTER H. WRIGHT
Pittsburgh

Quotation from a letter to the honorary editors by the retiring management.—"We have endeavored to make the *Journal of Dental Research*, since its establishment in 1919, an exemplar of the best and most meritorious in non-proprietary publication of dental research. The honorary editors have contributed the strength of their approval and support of that effort, and have responded to many calls for assistance. We cordially thank you for the very valuable aid you have given. Your interest and sympathy have fortified the *Journal's* determination to attain its avowed objectives. Your cooperation, accredited in each issue as a public endorsement of our purpose, has facilitated the *Journal's* growth and enhanced its usefulness. The *Journal*, on your recommendation, is about to pass into the complete control of the International Association for Dental Research, which will receive it as a gift from the original management. The Council has voted to place the *Journal* under the direction of a board of editors representative of, and to be selected by, the sections of the Association. Each of you, as a member of the Association, will have opportunity to participate in the recurrent selection of these editors. It is deeply gratifying to us, as we formally sever our past relationship, that the *Journal*, as the official publication of the Association, will continue to have the abiding interest and individual support of its former honorary editors."—*William J. Gies, Executive Officer, Board of Editors.*

INFORMATION FOR PROSPECTIVE SUBSCRIBERS OR CONTRIBUTORS

THE JOURNAL OF DENTAL RESEARCH is dedicated to the advancement of research, and to the dissemination of knowledge, pertaining to the teeth and mouth and to their relations to the body as a whole. It publishes original investigations and reviews in these fields, and in the sciences allied with or applied to them. Although without a "policy," the JOURNAL represents an unwavering purpose to keep widely open the channel to freedom of *responsible* and *respectable* expression of truth and opinion, by individuals or organizations, without qualification by or for any views its editors individually may entertain. Issued bi-monthly, at the *ends* of the months of February, April, June, August, October and December, each volume contains at least 500 pages, for which the subscription price is $5.00.

THE JOURNAL OF DENTAL RESEARCH circulates widely among the most enlightened and progressive dentists and physicians, and among the leading libraries, thus assuring the degree and quality of publicity for its contents that the best dental literature merits. Therefore there can be no important occasion, except in very special instances, for the detailed presentation elsewhere of a paper published in this JOURNAL. Accordingly, to prevent waste of the JOURNAL's resources in needless duplicate publication, and also to lighten the labors of students of the literature of dentistry, papers in this JOURNAL are not published in, or reprinted from, other dental journals, except for unusual professional reasons. Reprints in any number may be obtained at prices that will be quoted on request.

Address all communications relating to editorial matters to the JOURNAL OF DENTAL RESEARCH, 632 West 168th Street, New York City; business communications, to the JOURNAL OF DENTAL RESEARCH, Mount Royal and Guilford Avenues, Baltimore, Md.

WAVERLY PRESS, INC.
BALTIMORE, U. S. A.